Customer Number: 000959

DIVISIONAL-CONTINUATION APPLICATION TRANSMITTAL FORM UNDER RULE 1.53(b) (former Rule 1.60)

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION:		PRIOR APPLICATION SERIAL NUMBER: 08/456,104	PRIOR APPLICATION FILING DATE: MAY 30, 1995
RPI-008CPDV	CLASS:	SUBCLASS:	EXAMINER:	ART UNIT:

ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
WASHINGTON, DC 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: December 7, 1998 Mailing Label Number: EL094330961US

I hereby certify that this 37 CFR 1.53(b) request and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Viriato G. Cardoso — Viriato G. Cardoso
Name of Person Mailing Paper — Signature of Person Mailing Paper

Dear Sir:

This is a request for filing a ☐ continuation ☒ divisional application under 37 CFR 1.53(b), of pending prior application serial no. 08/456,104 filed on May 30, 1995, of Gordon J. Freeman, Lee M. Nadler and Gary S. Gray entitled Tumor Cells Modified To Express B7-2 With Increased Immunogenicity And Uses Therefor.

1. ☒ Enclosed is a copy of the latest inventor signed application, including the oath or declaration as originally filed. The copy of the enclosed papers is as follows:
   - ☒ 47 page(s) of specification
   - ☒ 7 page(s) of claims
   - ☒ 1 page(s) of abstract
   - ☒ 2 sheet(s) of drawing
   - ☒ ____ page(s) of declaration and power of attorney.

   I hereby verify that the attached papers are a true copy of the prior complete application serial no. 08/456,104 as originally filed on May 30, 1995.

2. ☒ The filing fee is calculated below:

	NUMBER OF CLAIMS FILED			NUMBER EXTRA
TOTAL	* 44	MINUS	** 20	= 24
INDEP.	* 8	MINUS	*** 3	= 5
☐ MULTIPLE DEPENDENT CLAIMS				

SMALL ENTITY			OTHER THAN A SMALL ENTITY	
RATE	FEE	OR	RATE	FEE
x 9 =	$.00		x 18 =	$ 432.00
x 39 =	$.00		x 78 =	$390.00
+130 =	$.00		+ 260 =	$.00
BASIC FEE	$.00		BASIC FEE	$760.00
TOTAL	$0.00	OR	TOTAL	$1582.00

3. ☐ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. ____. A duplicate copy of this sheet is enclosed.

4. ☒ Cancel in this application original claims 1-41, 44, and 45 of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

5. ☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claims in the prior application.)

6. ☒ Transfer the drawings from the pending prior application to this application.

7. ☒ The prior application is assigned of record to Dana-Farber Cancer Institute and Repligen Corporation.

8. ☒ The power of attorney in the prior application is to Lahive & Cockfield, LLP.

   a. ☒ The power appears in the original papers in the prior application.

   b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

   c. ☐ A new power has been executed and is attached.

9. ☒ A copy of An Associate Power of Attorney in USSN: 08/456,104 to Megan E. Williams is enclosed.

10. ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Amy E. Mandragouras at **Customer Number: 000959** whose address is:

Lahive & Cockfield, LLP
28 State Street
Boston, Massachusetts 02109

11. ☒ Any requests for extensions of time necessary in a parent application for establishing copendency between this application and a parent application are hereby requested and the Commissioner is authorized to charge any fee associated with such an extension to Deposit Account No. 12-0080.

12. ☒ Pursuant to 37 CFR 1.821(e), the computer readable form of the sequence listing for this new application is to be identical with the computer readable form of application serial no. 08/456,104. Please use the computer readable form of application serial no. 08/456,104 in lieu of filing a duplicate computer readable form in this application. Pursuant to 37 CFR 1.821(f), the content of the paper copy of the sequence listing for this new application and the computer readable form of application serial no. 08/456,104 are the same.

December 7, 1998
Date

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, Massachusetts 02109
Tel. (617) 227-7400

Megan E. Williams, Ph.D.
Reg. No. 43,270
☐ inventor(s) ☐ filed under §1.34(a)
☐ assignee of complete interest
☒ attorney or agent of record

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gordon J. Freeman, Lee M. Nadler and Gary S. Gray Serial No.: Not yet assigned Filed: December 7, 1998 For: Tumor Cells Modified to Express B7-2 with Increased Immunogenicity and Uses Therefor Attorney Docket No.: RPI-008CPDV	Group Art Unit: 1632 Examiner: K Hauda

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination, please amend the above-referenced patent application as follows:

In the Specification:

Please amend the specification as follows:

In the Title:

After "B7-2" please delete "and B7-3".

At page 1, line 11 under the Related Applications section please delete "This application is a Continuation-in-part of U.S. Serial No. 08/147,773 filed November 3, 1993 entitled 'Tumor Cells Modified to Express B7-2 and B7-3 with increased Immunogenicity and Uses Therefor'. The contents of this application is incorporated herein by reference." and insert therefore--This application is a continuation-in-part of USSN 08/456,104, filed on May 30, 1995, pending; which is a continuation-in-part of USSN 08/147,773, filed on

November 3, 1993, abandoned. The application also claims priority to PCT/US 94/08423, filed on July 26, 1994. The application also claims priority to USSN 08/280,757, filed on July 26, 1994, pending; which is a continuation-in-part of USSN 08/109,393, filed August 19, 1993, pending; which is a continuation-in-part of USSN 08/101,624, filed on July 26, 1993, abandoned. The contents of these applications are specifically incorporated herein by reference.--

In the Claims:

Please cancel claims 1-41 and 44-45 without prejudice.

Please amend the claims as follows:

46. (Amended) A method of treating a subject with a tumor, comprising:

(a) obtaining tumor cells from the subject;

(b) transfecting the tumor cells with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2 such that B7-2 is expressed by the tumor cell; and

(c) administering the tumor cells to the subject.

Please add the following new claims:

--65. The method of claim 46, wherein B7-2 comprises the amino acid sequence shown in SEQ ID NO:2.

66. The method of claim 46, wherein the nucleic acid encoding B7-2 molecule the nucleic sequence shown in SEQ ID NO:1.

67. The method of claim 58, wherein B7-2 molecule the amino acid sequence shown in SEQ ID NO:2.

68. The method of claim 58, wherein the nucleic acid encoding B7-2 comprises the nucleic sequence shown in SEQ ID NO:1.

69. The method of claim 60, wherein B7-2 comprises the amino acid sequence shown in SEQ ID NO:2.

70. The method of claim 60, wherein the nucleic acid encoding a B7-2 molecule comprises the nucleic sequence shown in SEQ ID NO:1.

71. The method of claim 62, wherein B7-2 comprises the amino acid sequence shown in SEQ ID NO:2.

72. The method of claim 62, wherein the nucleic acid encoding B7-2 comprises the nucleic sequence shown in SEQ ID NO:1.

73. A method of modifying a tumor cell to express a B7-2 molecule comprising, transfecting a tumor cell with a nucleic acid molecule encoding a B7-2 molecule such that B7-2 is expressed by the tumor cell.

74. The method of claim 73 wherein tumor cell is modified by transfection with a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1.

75. The method of claim 73, wherein the tumor cell is modified *in vitro* or *ex vivo.*

76. The method of claim 73, wherein the tumor cell is modified *in vivo.*

77. The method of claim 73, wherein the tumor cell is further transfected with at least one nucleic acid molecule encoding a B7 protein.

78. The method of claim 73 wherein the tumor cells are further transfected with at least one nucleic acid molecule encoding at least one MHC class II αchain protein and at least one MHC class II β chain protein in a form suitable for expression of the MHC class II αchain protein(s) and the MHC class II β chain protein(s).

79. The method of claim 73 wherein the tumor cells are further transfected with at least one nucleic acid molecule encoding at least one MHC class I α chain protein in a form suitable for expression of the MHC class I protein(s).

80. The method of claim 73 wherein the tumor cells are further transfected with a nucleic acid molecule encoding a β-2 microglobulin protein in a form suitable for expression of the β-2 microglobulin protein.

81. The method of claim 73 wherein expression of an MHC class II associated protein, the invariant chain, is inhibited in the tumor cells.

82. The method of claim 81 wherein expression of the invariant chain is inhibited in the tumor cells by transfection of the tumor cell with a nucleic acid which is antisense to a regulatory or a coding region of the invariant chain gene.

83. The method of claim 73 wherein the tumor is a sarcoma.

84. The method of claim 73 wherein the tumor is a lymphoma.

85. The method of claim 73 wherein the tumor is selected from a group consisting of a melanoma, a neuroblastoma, a leukemia and a carcinoma.

86. The method of claim 73, wherein the B7-2 molecule comprises the amino acid sequence shown in SEQ ID NO:2.

87. A method of increasing the immunogenecity of a tumor cell comprising, modifying the tumor cell to express a B7-2 T cell costimulatory molecule such that the immunogenecity of the tumor cell is increased.--

REMARKS

Claims 1-64 were present in parent application 08/456,104 as filed. Claims 1-41, 44 and 45 have been canceled. Claims 65-87 have been added. Accordingly, claims 42, 43, and 46-87 are currently pending in the application. For the Examiner's convenience, a copy of the claims as currently pending is provided in Appendix A.

Support for the above claim amendments can be found through out the specification and claims as originally filed. Support for the inclusion SEQ ID NO:s 1 and

2 in the claims can be found at least in the sequence listing as filed. No new matter has been added.

SUMMARY

If a telephone conversation with Applicants' Agent would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Agent at (617) 227-7400.

Respectfully submitted,

Megan E. Williams
Registration No. 43,270
Agent for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
(617) 227-5941

Dated: December 7, 1998

APPENDIX A

42. A method for treating a subject with a tumor, comprising:
   (a) obtaining tumor cells from the subject;
   (b) modifying the tumor cells to express B7-2, and
   (c) administering the tumor cells to the subject.

43. The method of claim 42 wherein tumor cells are modified by transfection with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2 .

46. A method of treating a subject with a tumor, comprising:
   (a) obtaining tumor cells from the subject;
   (b) transfecting the tumor cells with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2 such that B7-2 is expressed by the tumor cell; and
   (c) administering the tumor cells to the subject.

47. The method of claim 46 wherein the tumor cells are further transfected with a nucleic acid encoding B7.

48. The method of claim 46 wherein the tumor cells are further transfected with at least one nucleic acid encoding at least one MHC class II α chain protein and at least one MHC class II β chain protein in a form suitable for expression of the MHC class II α chain protein(s) and the MHC class II β chain protein(s).

49. The method of claim 46 wherein the tumor cells are further transfected with at least one nucleic acid encoding at least one MHC class I α chain protein in a form suitable for expression of the MHC class I protein(s).

50. The method of claim 49 wherein the tumor cells are further transfected with a nucleic acid encoding a β-2 microglobulin protein in a form suitable for expression of the β-2 microglobulin protein.

51. The method of claim 46 wherein expression of an MHC class II associated protein, the invariant chain, is inhibited in the tumor cells.

52. The method of claim 51 wherein expression of the invariant chain is inhibited in the tumor cells by transfection of the tumor cell with a nucleic acid which is antisense to a regulatory or a coding region of the invariant chain gene.

53. The method of claim 46 wherein the tumor is a sarcoma.

54. The method of claim 46 wherein the tumor is a lymphoma.

55. The method of claim 46 wherein the tumor is selected from a group consisting of a melanoma, a neuroblastoma, a leukemia and a carcinoma.

56. The method of claim 46 wherein the tumor cells are administered by intravenous injection.

57. The method of claim 46 wherein the tumor cells are administered by a route selected from a group consisting of intramuscular injection, intraperitoneal injection and subcutaneous injection.

58. A method for preventing or treating metastatic spread of a tumor or preventing or treating recurrence of a tumor in a subject, comprising:

(a) obtaining tumor cells from the subject;

(b) transfecting the tumor cells with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2; and

(c) administering the tumor cells to the subject.

59. The method of claim 58 wherein the tumor cells are further transfected with a nucleic acid encoding B7.

60. A method of inducing an anti-tumor response by CD4+ T lymphocytes in a subject with a tumor, comprising:

(a) obtaining tumor cells from the subject;

(b) transfecting the tumor cells with at least one nucleic acid comprising DNA encoding:

(i) B7-2,

(ii) an MHC class II α chain protein, and

(iii) an MHC class II β chain protein,

wherein the nucleic acid is in a form suitable for expression of B7-2, the MHC class II α chain protein and the MHC class II β chain protein; and
(c) administering the tumor cells to the subject.

61. A method for treating a subject with a tumor comprising modifying tumor cells *in vivo* to express a T cell costimulatory molecule, B7-2.

62. The method of claim 61 wherein tumor cells are modified *in vivo* by delivering to the subject *in vivo* a nucleic acid encoding B7-2 in a form suitable for expression of B7-2.

63. The method of claim 61 wherein the nucleic acid is delivered to the subject *in vivo* by injection of the nucleic acid in an appropriate vehicle into the tumor.

64. A method for treating a subject with a tumor, comprising:
(a) obtaining tumor cells and T lymphocytes from the subject;
(b) culturing the T lymphocytes from the subject *in vitro* with the tumor cells from the subject and with a stimulatory form of B7-2; and
(c) administering the T lymphocytes to the subject.

65. The method of claim 46, wherein B7-2 comprises the amino acid sequence shown in SEQ ID NO:2.

66. The method of claim 46, wherein the nucleic acid encoding B7-2 molecule the nucleic sequence shown in SEQ ID NO:1.

67. The method of claim 58, wherein B7-2 molecule the amino acid sequence shown in SEQ ID NO:2.

68. The method of claim 58, wherein the nucleic acid encoding B7-2 comprises the nucleic sequence shown in SEQ ID NO:1.

69. The method of claim 60, wherein B7-2 comprises the amino acid sequence shown in SEQ ID NO:2.

70. The method of claim 60, wherein the nucleic acid encoding a B7-2 molecule comprises the nucleic sequence shown in SEQ ID NO:1.

71. The method of claim 62, wherein B7-2 comprises the amino acid sequence shown in SEQ ID NO:2.

72. The method of claim 62, wherein the nucleic acid encoding B7-2 comprises the nucleic sequence shown in SEQ ID NO:1.

73. A method of modifying a tumor cell to express a B7-2 molecule comprising, transfecting a tumor cell with a nucleic acid molecule encoding a B7-2 molecule such that B7-2 is expressed by the tumor cell.

74. The method of claim 73 wherein tumor cell is modified by transfection with a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1.

75. The method of claim 73, wherein the tumor cell is modified *in vitro* or *ex vivo.*

76. The method of claim 73, wherein the tumor cell is modified *in vivo.*

77. The method of claim 73, wherein the tumor cell is further transfected with at least one nucleic acid molecule encoding a B7 protein.

78. The method of claim 73 wherein the tumor cells are further transfected with at least one nucleic acid molecule encoding at least one MHC class II αchain protein and at least one MHC class II β chain protein in a form suitable for expression of the MHC class II αchain protein(s) and the MHC class II β chain protein(s).

79. The method of claim 73 wherein the tumor cells are further transfected with at least one nucleic acid molecule encoding at least one MHC class I α chain protein in a form suitable for expression of the MHC class I protein(s).

80. The method of claim 73 wherein the tumor cells are further transfected with a nucleic acid molecule encoding a β-2 microglobulin protein in a form suitable for expression of the β-2 microglobulin protein.

81. The method of claim 73 wherein expression of an MHC class II associated protein, the invariant chain, is inhibited in the tumor cells.

82. The method of claim 81 wherein expression of the invariant chain is inhibited in the tumor cells by transfection of the tumor cell with a nucleic acid which is antisense to a regulatory or a coding region of the invariant chain gene.

83. The method of claim 73 wherein the tumor is a sarcoma.

84. The method of claim 73 wherein the tumor is a lymphoma.

85. The method of claim 73 wherein the tumor is selected from a group consisting of a melanoma, a neuroblastoma, a leukemia and a carcinoma.

86. The method of claim 73, wherein the B7-2 molecule comprises the amino acid sequence shown in SEQ ID NO:2.

87. A method of increasing the immunogenecity of a tumor cell comprising, modifying the tumor cell to express a B7-2 T cell costimulatory molecule such that the immunogenecity of the tumor cell is increased.

TUMOR CELLS MODIFIED TO EXPRESS B7-2 AND B7-3 WITH INCREASED IMMUNOGENICITY AND USES THEREFOR

Government Funding

Work described herein was supported under grant awarded by the National Institutes of Health. The U.S. government therefore may have certain rights to this invention.

Related Applications

This application is a Continuation-in-part of U.S. Serial No. 08/147,773 filed November 3, 1993 entitled "Tumor Cells Modified to Express B7-2 and B7-3 with Increased Immunogenicity and Uses Therefor". The contents of this application is incorporated herein by reference.

Background of the Invention

Induction of a T lymphocyte response is a critical initial step in a host's immune response. Activation of T cells results in T cell proliferation, cytokine production by T cells and generation of T cell-mediated effector functions. T cell activation requires an antigen-specific signal, often called a primary activation signal, which results from stimulation of a clonally-distributed T cell receptor (hereafter TcR) present on the surface of the T cell. This antigen-specific signal is usually in the form of an antigenic peptide bound either to a major histocompatibility complex (hereafter MHC) class I protein or an MHC class II protein present on the surface of an antigen presenting cell (hereafter APC). CD4+ T cells recognize peptides associated with class II molecules. Class II molecules are found on a limited number of cell types, primarily B cells, monocytes/macrophages and dendritic cells, and, in most cases, present peptides derived from proteins taken up from the extracellular environment. In contrast, CD8+ T cells recognize peptides associated with class I molecules. Class I molecules are found on almost all cell types and, in most cases, present peptides derived from endogenously synthesized proteins. For a review see Germain, R., *Nature* 322, 687-691 (1986).

It has now been established that, in addition to an antigen-specific primary activation signal, T cells also require a second, non-antigen specific, signal to induce full T cell proliferation and/or cytokine production. This phenomenon has been termed costimulation. Mueller, D.L., et al., *Annu. Rev. Immunol.* 7, 445-480 (1989). Like the antigen-specific signal, the costimulatory signal is triggered by a molecule on the surface of the antigen presenting cell. A costimulatory molecule, the B lymphocyte antigen B7, has been identified on activated B cells and other APCs. Freeman, G.J., et al., *J. Immunol.* 139, 3260-3267 (1987); Freeman, G.J., et al., *J. Immunol.* 143, 2714-2722 (1989). Binding of B7 to a ligand

on the surface of T cells provides costimulation to the T cell. Two structurally similar T cell-surface receptors for B7 have been identified, CD28 and CTLA-4. Aruffo, A. and Seed, B., *Proc. Natl. Acad. Sci. USA* 84, 8573-8577 (1987); Linsley, P.S., et al., *J. Exp. Med.* 173, 721-730, (1991); Brunet, J.F., et al., *Nature* 328, 267-270 (1987); Brunet, J.F., et al., *Immunol Rev.* 103, 21-36 (1988). CD28 is expressed constitutively on T cells and its expression is upregulated by activation of the T cell, such as by interaction of the TcR with an antigen-MHC complex. In contrast, CTLA4 is undetectable on resting T cells and its expression is induced by activation.

A series of experiments have shown a functional role for a T cell activation pathway stimulated through the CD28 receptor. Studies using blocking antibodies to B7 and CD28 have demonstrated that these antibodies can inhibit T cell activation, thereby demonstrating the need for stimulation via this pathway for T cell activation. Furthermore, suboptimal polyclonal stimulation of T cells by phorbol ester or anti-CD3 antibodies can be potentiated by crosslinking of CD28 with anti-CD28 antibodies. Engagement of the TcR by an MHC molecule/peptide complex in the absence of the costimulatory B7 signal can lead to T cell anergy rather than activation. Damle, N.K., et al., *Proc. Natl. Acad. Sci. USA* 78, 5096-5100 (1981); Lesslauer, W., et al., *Eur. J. Immunol.* 16, 1289-1295 (1986); Gimmi, C.D., et al., *Proc. Natl. Acad. Sci. USA* 88, 6575-6579 (1991); Linsley, P.S., et al., *J. Exp. Med.* 173; 721-730 (1991); Koulova, L., et al., *J. Exp. Med.* 173, 759-762 (1991); Razi-Wolf, Z., et al., *Proc. Natl. Acad. Sci. USA* 89, 4210-4214 (1992).

Malignant transformation of a cell is commonly associated with phenotypic changes in the cell. Such changes can include loss or gain of expression of some proteins or alterations in the level of expression of certain proteins. It has been hypothesized that in some situations the immune system may be capable of recognizing a tumor as foreign and, as such, could mount an immune response against the tumor. Kripke, M., *Adv. Cancer Res.* 34, 69-75 (1981). This hypothesis is based in part on the existence of phenotypic differences between a tumor cell and a normal cell, which is supported by the identification of tumor associated antigens (hereafter TAAs). Schreiber, H., et al. *Ann. Rev. Immunol.* 6, 465-483 (1988). TAAs are thought to distinguish a transformed cell from its normal counterpart. Three genes encoding TAAs expressed in melanoma cells, MAGE-1, MAGE-2 and MAGE-3, have recently been cloned. van der Bruggen, P., et al. *Science* 254, 1643-1647 (1991). That tumor cells under certain circumstances can be recognized as foreign is also supported by the existence of T cells which can recognize and respond to tumor associated antigens presented by MHC molecules. Such TAA-specific T lymphocytes have been demonstrated to be present in the immune repertoire and are capable of recognizing and stimulating an immune response against tumor cells when properly stimulated *in vitro*. Rosenberg, S.A., et al. *Science* 233, 1318-1321 (1986); Rosenberg, S.A. and Lotze, M.T. *Ann. Rev. Immunol.* 4, 681-709 (1986).

However, in practice, tumors *in vivo* have generally not been found to be very immunogenic and appear to be capable of evading immune response. This may result from an inability of tumor cells to induce T cell-mediated immune responses. Ostrand-Rosenberg, S., et al., *J. Immunol.* 144, 4068-4071 (1990); Fearon, E.R., et al., *Cell* 60, 397-403 (1990). A method for increasing the immunogenicity of a tumor cell *in vivo* would be therapeutically beneficial.

Summary of the Invention

Although most tumor cells are thought to express TAAs which distinguish tumor cells from normal cells and T cells which recognize TAA peptides have been identified in the immune repertoire, an anti-tumor T cell response may not be induced by a tumor cell due to a lack of costimulation necessary to activate the T cells. It is known that many tumors are derived from cells which do not normally function as antigen-presenting cells, and, thus, may not trigger necessary signals for T cell activation. In particular, tumor cells may be incapable of triggering a costimulatory signal in a T cell which is required for activation of the T cell. This invention is based, at least in part, on the discovery that tumor cells modified to express a costimulatory molecule, and therefore capable of triggering a costimulatory signal, can induce an anti-tumor T cell-mediated immune response *in vivo*. This T cell-mediated immune response is effective not only against the modified tumor cells but, more importantly, against the unmodified tumor cells from which they were derived. Thus, the effector phase of the anti-tumor response induced by the modified tumor cells of the invention is not dependent upon expression of a costimulatory molecule on the tumor cells.

Accordingly, the invention pertains to methods of inducing or enhancing T lymphocyte-mediated anti-tumor immunity in a subject by use of a modified tumor cell having increased immunogenicity. In one aspect of the invention, a tumor cell is modified to express one or more T cell costimulatory molecules on its surface. Preferred costimulatory molecules are novel B lymphocyte antigens, B7-2 and B7-3. Prior to modification, the tumor cell may lack the ability to express B7-2 and/or B7-3, may be capable of expressing B7-2 and/or B7-3 but fail to do so, or may express insufficient amounts of B7-2 and/or B7-3 to activate T cells. Therefore, a tumor cell can be modified by providing B7-2 and/or B7-3 to the tumor cell surface, by inducing the expression of B7-2 and/or B7-3 on the tumor cell or by increasing the level of expression of B7-2 and/or B7-3 on the tumor cell. In one embodiment, the tumor cell is modified by transfecting the cell with at least one nucleic acid encoding B7-2 and/or B7-3 in a form suitable for expression of the molecule(s) on the cell surface. Alternatively, the tumor cell is contacted with an agent which induces or increases expression of B7-2 and/or B7-3 on the cell surface. In yet another embodiment, the tumor cell is modified by chemically coupling B7-2 and/or B7-3 to the tumor cell surface. A tumor cell modified to express B7-2 and/or B7-3 can be further modified to express the T cell costimulatory molecule B7.

Even when provided with the ability to trigger a costimulatory signal in T cells, modified tumor cells may still be incapable of inducing anti-tumor T cell-mediated immune responses due to a failure to sufficiently trigger an antigen-specific primary activation signal. This can result from insufficient expression of MHC class I or class II molecules on the tumor cell surface. Accordingly, this invention encompasses modified tumor cells which provide both a T cell costimulatory signal and an antigen-specific primary activation signal, via an antigen-MHC complex, to T cells. Prior to modification, a tumor cell may lack the ability to express one or more MHC molecules, may be capable of expressing one or more MHC molecules but fail to do so, may express only certain types of MHC molecules (e.g., class I but not class II), or may express insufficient amounts of MHC molecules to activate T cells. Thus, in one embodiment, a tumor cell is modified by providing one or more MHC molecules to the tumor cell surface, by inducing the expression of one or more MHC molecules on the tumor cell surface or by increasing the level of expression of one or more MHC molecules on the tumor cell surface. Tumor cells expressing B7-2 and/or B7-3 are further modified, for example, by transfection with a nucleic acid encoding one or more MHC molecules in a form suitable for expression of the MHC molecule(s) on the tumor cell surface. Alternatively, such tumor cells are modified by contact with an agent which induces or increases expression of one or more MHC molecules on the cell.

In a particularly preferred embodiment, tumor cells modified to express B7-2 and/or B7-3 are further modified to express one or more MHC class II molecules. To provide an MHC class II molecule, at least one nucleic acid encoding an MHC class II α chain protein and an MHC class II β chain protein are introduced into the tumor cell such that expression of these proteins is directed to the surface of the cell. In yet another embodiment, tumor cells modified to express B7-2 and/or B7-3 are further modified to express one or more MHC class I molecules. To provide an MHC class I molecule, at least one nucleic acid encoding an MHC class I α chain protein and a β-2 microglobulin protein are introduced such that expression of these proteins is directed to the surface of the tumor cell. Alternatively, a tumor cell modified to express B7-2 and/or B7-3 can be further modified by contact with an agent which induces or increases the expression of MHC molecules (class I and/or class II) on the cell surface.

In certain situations, modified tumor cells of the invention may fail to activate T cells because of insufficient association of TAA-derived peptides with MHC molecules, resulting in a lack of an antigen-specific primary activation signal in T cells. Accordingly, the invention further pertains to a tumor cell modified to trigger a costimulatory signal in T cells and in which association of TAA peptides with MHC class II molecules is promoted in order to induce an antigen-specific signal in T cells. This aspect of the invention is based, at least in part, on the ability of an MHC class II associated protein, the invariant chain, to prevent association of endogenously derived peptides (which would include a number of TAA peptides) with MHC class II molecules intracellularly. Thus, in one embodiment, a tumor

cell modified to express B7-2 and/or B7-3 is further modified to promote association of TAA peptides with MHC class II molecules by inhibiting the expression of the invariant chain in the tumor cell. The tumor cell selected to be so modified can be one which naturally expresses both MHC class II molecules and the invariant chain or can be one which expresses the invariant chain and which has been modified to express MHC class II molecules. Preferably, expression of the invariant chain is inhibited in a tumor cell by introducing into the tumor cell a nucleic acid which is antisense to a coding or regulatory region of the invariant chain gene. Alternatively, expression of the invariant chain in a tumor cell is prevented by an agent which inhibits expression of the invariant chain gene or which inhibits expression or activity of the invariant chain protein.

The modified tumor cells of the invention can be used in methods for inducing an anti-tumor T lymphocyte response in a subject effective against both modified and unmodified tumor cells. For example, tumor cells can be obtained, modified as described herein to trigger a costimulatory signal in T lymphocytes, and administered to the subject to elicit a T cell-mediated immune response. The modified tumor cells of the invention can also be administered to prevent or inhibit metastatic spread of a tumor or to prevent or inhibit recurrence of a tumor following therapeutic treatment.

This invention also provides methods for treating a subject with a tumor by modifying tumor cells *in vivo* to be capable of triggering a costimulatory signal in T cells, and, if necessary, also an antigen-specific signal.

The tumor cells of the current invention modified to express B7-2 and/or B7-3 and one or more MHC class II molecules can be used in a method for specifically inducing an anti-tumor response by CD4+ T lymphocytes in a subject with a tumor by administering the modified tumor cells to the subject. Alternatively, a CD4+ T cell response can be induced by modifying tumor cells *in vivo* to express a B7-2 and/or B7-3 and one or more MHC class II molecules.

The invention also pertains to a composition of modified tumor cells suitable for pharmaceutical administration. This composition comprises an amount of tumor cells and a physiologically acceptable carrier.

Brief Description of the Drawings

Figure 1 shows graphs depicting the cell surface expression of B7 and the MHC class II molecule I-A^k on wild -type and transfected tumor cells as determined by immunofluourescent staining of the cells.

Figure 2 is a graphic representation of tumor cell growth (as measured by tumor size) in mice following transplantation of J558 plasmacytoma cells or J558 plasmacytoma cells transfected to express B7-1 (J558-B7.1) or B7-2 (J558-B7.2).

Detailed Description of the Invention

The induction of a T cell response requires that at least two signals be delivered by ligands on a stimulator cell to the T cell through cell surface receptors on the T cell. A primary activation signal is delivered to the T cell through the antigen-specific TcR. Physiologically, this signal is triggered by an antigen-MHC molecule complex on the stimulator cell, although it can also be triggered by other means such as phorbol ester treatment or crosslinking of the TcR complex with antibodies, e.g. with anti-CD3. To induce T cell activation, a second signal, called a costimulatory signal, is required by stimulation of the T cell through another cell surface molecule, such as CD28 or CTLA4. Thus, the minimal molecules on a stimulator cell required for T cell activation are an MHC molecule associated with a peptide antigen, to trigger a primary activation signal in a T cell, and a costimulatory molecule to trigger a costimulatory signal in the T cell. Engagement of the antigen-specific TcR in the absence of triggering of a costimulatory signal can prevent activation of the T cell and, in addition, can induce a state of unresponsiveness or anergy in the T cells.

In addition to the previously characterized B lymphocyte activation antigen B7, human B lymphocytes express other novel molecules which costimulate T cell activation. These costimulatory molecules include antigens on the surface of B lymphocytes, professional antigen presenting cells (e.g., monocytes, dendritic cells, Langerhan cells) and other cells which present antigen to immune cells (e.g., keratinocytes, endothelial cells, astrocytes, fibroblasts, oligodendrocytes) and which bind either CTLA4, CD28, both CTLA4 and CD28 or other known or as yet undefined receptors on immune cells. Novel B lymphocyte antigens which provide cotimulation to activated T cells to thereby induce T cell proliferation and/or cytokine secretion include the B7-2 (human and mouse) and the B7-3 antigens described herein.

The B lymphocyte antigen B7-2 is expressed by human B cells at about 24 hours following stimulation with either anti-immunoglobulin or anti-MHC class II monoclonal antibody. The B7-2 antigen induces detectable IL-2 secretion and T cell proliferation. At about 48 to 72 hours post activation, human B cells express both B7 and a third CTLA4 counter-receptor, B7-3, identified by a monoclonal antibody BB-1, which also binds B7 (Yokochi, T., et al. (1982) *J. Immunol.* 128, 823-827). The B7-3 antigen is also expressed on B7 negative activated B cells and can costimulate T cell proliferation without detectable IL-2 production, indicating that the B7 and B7-3 molecules are distinct. B7-3 is expressed on a wide variety of cells including activated B cells, activated monocytes, dendritic cells, Langerhan cells and keratinocytes. At 72 hours post B cell activation, the expression of B7 and B7-3 begins to decline. The presence of these costimulatory molecules on the surface of activated B lymphocytes indicates that T cell costimulation is regulated, in part, by the temporal expression of these molecules following B cell activation.

The ability of a tumor cell to evade an immune response and fail to stimulate a T lymphocyte response against the cell may result from the inability of the cell to properly activate T cells. This invention provides modified tumor cells which trigger a costimulatory signal in T cells and, thus, activate an anti-tumor T lymphocyte response. Tumor cells are modified to be capable of triggering a costimulatory signal by providing B7-2 and/or B7-3 to the tumors. Tumors cells may be further modified by providing B7. Additionally, in certain embodiments, tumor cells are modified to trigger both a primary, antigen-specific activation signal and a costimulatory signal in T cells.

The modified tumor cells of the invention display increased immunogenicity and can be used to induce or enhance a T cell-mediated immune response against a tumor. Since the effector phase of the T cell-mediated immune response is not dependent upon expression of a costimulatory molecule by tumor cells, the T cell-mediated immune response generated by administration of a modified tumor cell of the invention is effective against not only the modified tumor cells but also the unmodified tumor cells from which they were derived.

I. *Ex Vivo* Modification of a Tumor Cell to Express a Costimulatory Molecule

The inability of a tumor cell to trigger a costimulatory signal in T cells may be due to a lack of expression of a costimulatory molecule, failure to express a costimulatory molecule even though the tumor cell is capable of expressing such a molecule, insufficient expression of a costimulatory molecule on the tumor cell surface or lack of expression of an appropriate costimulatory molecule (e.g. expression of B7 but not B7-2 and/or B7-3). Thus, according to one aspect of the invention, a tumor cell is modified to express B7-2 and/or B7-3 by transfection of the tumor cell with a nucleic acid encoding B7-2 and/or B7-3 in a form suitable for expression of B7-2 and/or B7-3 on the tumor cell surface. Alternatively, the tumor cell is modified by contact with an agent which induces or increases expression of B7-2 and/or B7-3 on the tumor cell surface. In yet another embodiment, B7-2 and/or B7-3 is coupled to the surface of the tumor cell to produce a modified tumor cell.

The ability of a molecule, such as B7-2 or B7-3, to provide a costimulatory signal to T cells can be determined, for example, by contacting T cells which have received a primary activation signal with the molecule to be tested and determining the presence of T cell proliferation and/or cytokine secretion. T cell can be suboptimally stimulated with a primary activation signal, for instance by contact with immobilized anti-CD3 antibodies or a phorbol ester. Following this stimulation, the T cells are exposed to cells expressing B7-2 and/or B7-3 on their surface and the proliferation of the T cells and/or secretion of cytokines, such as IL-2, by the T cells is determined. Proliferation and/or cytokine secretion will be increased by triggering of a costimulatory signal in the T cells. T cell proliferation can be measured, for example, by a standard ^{3}H-thymidine uptake assay. Cytokine secretion can be measured, for example, by a standard IL-2 assay. See for example Linsley, P.S., et al., *J. Exp. Med.* 173, 721-730 (1991), Gimmi, C.D., et al., *Proc. Natl. Acad. Sci. USA* 88:, 6575-6579 (1991), Freeman, G.J., et al., *J. Exp. Med.* 174, 625-631, (1991).

Fragments, mutants or variants of B7-2 and/or B7-3 that retain the ability to interact with T cells, trigger a costimulatory signal and activate T cell responses, as evidenced by proliferation and/or cytokine production by T cells that have received a primary activation signal, are considered within the scope of the invention. A "fragment" of B7-2 and/or B7-3 is defined as a portion of B7-2 and/or B7-3 which retains costimulatory activity. For example, a fragment of B7-2 and/or B7-3 may have fewer amino acid residues than the entire protein. A "mutant" is defined as B7-2 and/or B7-3 having a structural change which may enhance, diminish, not affect, but not eliminate the costimulatory activity of the molecule. For example, a mutant of B7-2 and/or B7-3 may have a change in one or more amino acid residues of the protein. A "variant" is defined as B7-2 and/or B7-3 having a modification which does not affect the costimulatory activity of the molecule. For example, a variant of B7-2 and/or B7-3 may have altered glycosylation or may be a chimeric protein of the costimulatory molecule and another protein.

A. Transfection of a Tumor Cell with a Nucleic Acid Encoding a Costimulatory Molecule

Tumor cells can be modified *ex vivo* to express B7-2 and/or B7-3 by transfection of isolated tumor cells with a nucleic acid encoding B7-2 and/or B7-3 in a form suitable for expression of the molecule on the surface of the tumor cell. The terms "transfection" or "transfected with" refers to the introduction of exogenous nucleic acid into a mammalian cell and encompass a variety of techniques useful for introduction of nucleic acids into mammalian cells including electroporation, calcium-phosphate precipitation, DEAE-dextran treatment, lipofection, microinjection and infection with viral vectors. Suitable methods for transfecting mammalian cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)) and other laboratory textbooks. The nucleic acid to be introduced may be, for example, DNA encompassing the gene(s) encoding B7-2 and/or B7-3, sense strand RNA encoding B7-2 and/or B7-3 or a recombinant expression vector containing a cDNA encoding B7-2 and/or B7-3. The nucleotide sequence of a cDNA encoding human B7-2 is shown in SEQ ID NO: 1, and the amino acid sequence of a human B7-2 protein is shown in SEQ ID NO:2. The nucleotide sequence of a cDNA encoding mouse B7-2 is shown in SEQ ID NO: 3, and the amino acid sequence of a mouse B7-2 protein is shown in SEQ ID NO:4.

The nucleic acid is "in a form suitable for expression of B7-2" or "in a form suitable for expression of B7-3" in which the nucleic acid contains all of the coding and regulatory sequences required for transcription and translation of a gene, which may include promoters, enhancers and polyadenylation signals, and sequences necessary for transport of the molecule to the surface of the tumor cell, including N-terminal signal sequences. When the nucleic acid is a cDNA in a recombinant expression vector, the regulatory functions responsible for transcription and/or translation of the cDNA are often provided by viral sequences. Examples of commonly used viral promoters include those derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40, and retroviral LTRs. Regulatory sequences linked to the cDNA can be selected to provide constitutive or inducible transcription, by, for example, use of an inducible promoter, such as the metallothienin promoter or a glucocorticoid-responsive promoter. Expression of B7-2 or B7-3 on the surface of the tumor cell can be accomplished, for example, by including the native trans-membrane coding sequence of the molecule in the nucleic acid sequence, or by including signals which lead to modification of the protein, such as a C-terminal inositol-phosphate linkage, that allows for association of the molecule with the outer surface of the cell membrane.

A preferred approach for introducing nucleic acid encoding B7-2 and/or B7-3 into tumor cells is by use of a viral vector containing nucleic acid, e.g. a cDNA, encoding B7-2 and/or B7-3. Examples of viral vectors which can be used include retroviral vectors (Eglitis, M.A., et al., *Science* 230, 1395-1398 (1985); Danos, O. and Mulligan, R., *Proc. Natl. Acad. Sci. USA* 85; 6460-6464 (1988); Markowitz, D., et al., *J. Virol.* 62, 1120-1124 (1988)),

adenoviral vectors (Rosenfeld, M.A., et al., *Cell* 68, 143-155 (1992)) and adeno-associated viral vectors (Tratschin, J.D., et al., *Mol. Cell. Biol.* 5, 3251-3260 (1985)). Infection of tumor cells with a viral vector has the advantage that a large proportion of cells will receive nucleic acid, thereby obviating a need for selection of cells which have received nucleic acid, and molecules encoded within the viral vector, e.g. by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

Alternatively, B7-2 and/or B7-3 can be expressed on a tumor cell using a plasmid expression vector which contains nucleic acid, e.g. a cDNA, encoding B7-2 and/or B7-3. Suitable plasmid expression vectors include CDM8 (Seed, B., *Nature* 329, 840 (1987)) and pMT2PC (Kaufman, et al., *EMBO J.* 6, 187-195 (1987)). Since only a small fraction of cells (about 1 out of 10^5) typically integrate transfected plasmid DNA into their genomes, it is advantageous to transfect a nucleic acid encoding a selectable marker into the tumor cell along with the nucleic acid(s) of interest. Preferred selectable markers include those which confer resistance to drugs such as G418, hygromycin and methotrexate. Selectable markers may be introduced on the same plasmid as the gene(s) of interest or may be introduced on a separate plasmid. Following selection of transfected tumor cells using the appropriate selectable marker(s), expression of the costimulatory molecule on the surface of the tumor cell can be confirmed by immunofluorescent staining of the cells. For example, cells may be stained with a fluorescently labeled monoclonal antibody reactive against the costimulatory molecule or with a fluorescently labeled soluble receptor which binds the costimulatory molecule. Expression of the B7-3 costimulatory molecule can be determined using a monoclonal antibody, BB1, which recognizes B7-3. Yokochi, T., et al. *J. Immunol.* 128, 823-827 (1982). Alternatively, a labeled soluble CD28 or CTLA4 protein or fusion protein (e.g. CTLA4Ig) which binds to B7-2 and B7-3 can be used to detect expression of B7-2 and/or B7-3.

When transfection of tumor cells leads to modification of a large proportion of the tumor cells and efficient expression of B7-2 and/or B7-3 on the surface of tumor cells, e.g. when using a viral expression vector, tumor cells may be used without further isolation or subcloning. Alternatively, a homogenous population of transfected tumor cells can be prepared by isolating a single transfected tumor cell by limiting dilution cloning followed by expansion of the single tumor cell into a clonal population of cells by standard techniques.

B. Induction or Increased Expression of a Costimulatory Molecule on a Tumor Cell Surface

A tumor cell can be modified to trigger a costimulatory signal in T cells by inducing or increasing the level of expression of B7-2 and/or B7-3 on a tumor cell which is capable of expressing B7-2 and/or B7-3 but fails to do so or which expresses insufficient amounts of B7-2 and/or B7-3 to activate T cells. An agent which stimulates expression of B7-2 and/or B7-3 can be used in order to induce or increase expression of B7-2 and/or B7-3 on the tumor cell surface. For example, tumor cells can be contacted with the agent *in vitro* in a culture

medium. The agent which stimulates expression of B7-2 and/or B7-3 may act, for instance, by increasing transcription of B7-2 and/or B7-3 gene, by increasing translation of B7-2 and/or B7-3 mRNA or by increasing stability or transport of B7-2 and/or B7-3 to the cell surface. For example, it is known that expression of B7 can be upregulated in a cell by a second messenger pathway involving cAMP. Nabavi, N., et al. *Nature* 360, 266-268 (1992). B7-2 and B7-3 may likewise be inducible by cAMP. Thus, a tumor cell can be contacted with an agent, which increases intracellular cAMP levels or which mimics cAMP, such as a cAMP analogue, e.g. dibutyryl cAMP, to stimulate expression of B7-2 and/or B7-3 on the tumor cell surface. It is also known that expression of B7 can be induced on normal resting B cells by crosslinking cell-surface MHC class II molecules on the B cells with an antibody against the MHC class II molecules. Kuolova, L., et al., *J. Exp. Med.* 173, 759-762 (1991). Similarly, B7-2 and B7-3 can be induced on resting B cells by crosslinking cell-surface MHC class II molecules on the B cells. Accordingly, a tumor cell which expresses MHC class II molecules on its surface can be treated with anti-MHC class II antibodies to induce or increase B7-2 and or B7-3 expression on the tumor cell surface.

Another agent which can be used to induce or increase expression of B7-2 and/or B7-3 on a tumor cell surface is a nucleic acid encoding a transcription factor which upregulates transcription of the gene encoding the costimulatory molecule. This nucleic acid can be transfected into the tumor cell to cause increased transcription of the costimulatory molecule gene, resulting in increased cell-surface levels of the costimulatory molecule.

C. Coupling of a Costimulatory Molecule to the Surface of a Tumor Cell

In another embodiment, a tumor cell is modified to be capable of triggering a costimulatory signal in T cells by coupling B7-2 and/or B7-3 to the surface of the tumor cell. For example, B7-2 and/or B7-3 molecules can be obtained using standard recombinant DNA technology and expression systems which allow for production and isolation of the costimulatory molecule(s). Alternatively, B7-2 and/or B7-3 can be isolated from cells which express the costimulatory molecule(s) using standard protein purification techniques. For example, B7-3 protein can be isolated from activated B cells by immunoprecipitation with an anti-B7-3 antibody such as the BB1 monoclonal antibody. The isolated costimulatory molecule is then coupled to the tumor cell. The terms "coupled" or "coupling" refer to a chemical, enzymatic or other means (e.g., antibody) by which B7-2 and/or B7-3 is linked to a tumor cell such that the costimulatory molecule is present on the surface of the tumor cell and is capable of triggering a costimulatory signal in T cells. For example, B7-2 and/or B7-3 can be chemically crosslinked to the tumor cell surface using commercially available crosslinking reagents (Pierce, Rockford IL). Another approach to coupling B7-2 and/or B7-3 to a tumor cell is to use a bispecific antibody which binds both the costimulatory molecule and a cell-surface molecule on the tumor cell. Fragments, mutants or variants of B7-2 and/or B7-3

which retain the ability to trigger a costimulatory signal in T cells when coupled to the surface of a tumor cell can also be used.

D. Modification of Tumor Cells to Express Multiple Costimulatory Molecules

Another aspect of the invention is a tumor cell modified to express multiple costimulatory molecules. The temporal expression of costimulatory molecules on activated B cells is different for B7, B7-2 and B7-3. For example, B7-2 is expressed early following B cell activation, whereas B7-3 is expressed later. The different costimulatory molecules may thus serve distinct functions during the course of an immune response. An effective T cell response may require that the T cell receive costimulatory signals from multiple costimulatory molecules. Accordingly, the invention encompasses a tumor cell which is modified to express more than one costimulatory molecule. For example, a tumor cell can be modified to express both B7-2 and B7-3. Alternatively, a tumor cell modified to express B7-2 can be further modified to express B7. Similarly, a tumor cell modified to express B7-3 can be further modified to express B7. A tumor cell can also be modified to express B7, B7-2 and B7-3.

Before modification, a tumor cell may not express any costimulatory molecules, or may express certain costimulatory molecules but not others. As described herein, tumor cells can be modified by transfecting the tumor cell with nucleic acid encoding a costimulatory molecule(s), by inducing the expression of a costimulatory molecule(s) or by coupling a costimulatory molecule(s) to the tumor cell. For example, a tumor cell transfected with nucleic acid encoding B7-2 can be further transfected with nucleic acid encoding B7. The cDNA sequence and deduced amino acid sequence of human and mouse B7 is shown in SEQ ID NO:5 and 6 and SEQ ID NO:7 and 8, respectively. Alternatively, more than one type of modification can be used. For example, a tumor cell transfected with a nucleic acid encoding B7-2 can be stimulated with an agent which induces expression of B7.

II. Additional Modification of a Tumor Cell to Express MHC Molecules

Another aspect of this invention features modified tumor cells which express a costimulatory molecule and which express one or more MHC molecules on their surface to trigger both a costimulatory signal and a primary, antigen-specific, signal in T cells. Before modification, tumor cells may be unable to express MHC molecules, may fail to express MHC molecules although they are capable of expressing such molecules, or may express insufficient amounts of MHC molecules on the tumor cell surface to cause T cell activation. Tumor cells can be modified to express either MHC class I or MHC class II molecules, or both. One approach to modifying tumor cells to express MHC molecules is to transfect the tumor cell with one or more nucleic acids encoding one or more MHC molecules. Alternatively, an agent which induces or increases expression of one or more MHC molecules on tumor cells can be used to modify tumor cells. Inducing or increasing

expression of MHC class II molecules on a tumor cell can be particularly beneficial for activating $CD4^+$ T cells against the tumor since the ability of MHC class II^+ tumor cells to directly present tumor peptides to $CD4^+$ T cells bypasses the need for professional MHC class II^+ APCs. This can improve tumor immunogenicity because soluble tumor antigen (in the form of tumor cell debris or secreted protein) may not be available for uptake by professional MHC class II^+ APCs.

One embodiment of the invention is a modified tumor cell which expresses B7-2 and/or B7-3 and one or more MHC class II molecules on their cell surface. MHC class II molecules are cell-surface α/β heterodimers which structurally contain a cleft into which antigenic peptides bind and which function to present bound peptides to the antigen-specific TcR. Multiple, different MHC class II proteins are expressed on professional APCs and different MHC class II proteins bind different antigenic peptides. Expression of multiple MHC class II molecules, therefore, increases the spectrum of antigenic peptides that can be presented by an APC or by a modified tumor cell. The α and β chains of MHC class II molecules are encoded by different genes. For instance, the human MHC class II protein HLA-DR is encoded by the HLA-DRα and HLA-DRβ genes. Additionally, many polymorphic alleles of MHC class II genes exist in human and other species. T cells of a particular individual respond to stimulation by antigenic peptides in conjunction with self MHC molecules, a phenomenon termed MHC restriction. In addition, certain T cells can also respond to stimulation by polymorphic alleles of MHC molecules found on the cells of other individuals, a phenomenon termed allogenicity. For a review of MHC class II structure and function, see Germain and Margulies, *Ann. Rev. Immunol.* 11: 403-450, 1993.

Another embodiment of the invention is a modified tumor cell which expresses B7-2 and/or B7-3 and one or more MHC class I molecules on the cell surface. Similar to MHC class II genes, there are multiple MHC class I genes and many polymorphic alleles of these genes are found in human and other species. Like MHC class II proteins, class I proteins bind peptide fragments of antigens for presentation to T cells. A functional cell-surface class I molecule is composed of an MHC class I α chain protein associated with a β2-microglobulin protein.

A. Transfection of a Tumor Cell with Nucleic Acid Encoding MHC Molecules

Tumor cells can be modified *ex vivo* to express one or more MHC class II molecules by transfection of isolated tumor cells with one or more nucleic acids encoding one or more MHC class II α chains and one or more MHC class II β chains in a form suitable for expression of the MHC class II molecules(s) on the surface of the tumor cell. Both an α and a β chain protein must be present in the tumor cell to form a surface heterodimer and neither chain will be expressed on the cell surface alone. The nucleic acid sequences of many murine and human class II genes are known. For examples see Hood, L., et al. *Ann. Rev. Immunol.* 1, 529-568 (1983) and Auffray, C. and Strominger, J.L., *Advances in Human Genetics* 15, 197-

247 (1987). Preferably, the introduced MHC class II molecule is a self MHC class II molecule. Alternatively, the MHC class II molecule could be a foreign, allogeneic, MHC class II molecule. A particular foreign MHC class II molecule to be introduced into tumor cells can be selected by its ability to induce T cells from a tumor-bearing subject to proliferate and/or secrete cytokines when stimulated by cells expressing the foreign MHC class II molecule (i.e. by its ability to induce an allogeneic response). The tumor cells to be transfected may not express MHC class II molecules on their surface prior to transfection or may express amounts insufficient to stimulate a T cell response. Alternatively, tumor cells which express MHC class II molecules prior to transfection can be further transfected with additional, different MHC class II genes or with other polymorphic alleles of MHC class II genes to increase the spectrum of antigenic fragments that the tumor cells can present to T cells.

Fragments, mutants or variants of MHC class II molecules that retain the ability to bind peptide antigens and activate T cell responses, as evidenced by proliferation and/or lymphokine production by T cells, are considered within the scope of the invention. A preferred variant is an MHC class II molecule in which the cytoplasmic domain of either one or both of the α and β chains is truncated. It is known that truncation of the cytoplasmic domains allows peptide binding by and cell surface expression of MHC class II molecules but prevents the induction of endogenous B7 expression, which is triggered by an intracellular signal generated by the cytoplasmic domains of the MHC class II protein chains upon crosslinking of cell surface MHC class II molecules. Kuolova. L., et al., *J. Exp. Med.* 173, 759-762 (1991); Nabavi, N., et al. *Nature* 360, 266-268 (1992). Expression of B7-2 and B7-3 is also induced by crosslinking surface MHC class II molecules, and thus truncation of MHC class II molecules may also prevent induction of B7-2 and/or B7-3. In tumor cells transfected to constitutively express B7-2 and/or B7-3, it may be desirable to inhibit the expression of endogenous costimulatory molecules, for instance to restrain potential downregulatory feedback mechanisms. Transfection of a tumor cell with a nucleic acid(s) encoding a cytoplasmic domain-truncated form of MHC class II α and β chain proteins would inhibit endogenous B7 expression and possibly also endogenous B7-2 and B7-3 expression. Such variants can be produced by, for example, introducing a stop codon in the MHC class II chain gene(s) after the nucleotides encoding the transmembrane spanning region. The cytoplasmic domain of either the α chain or the β chain protein can be truncated, or, for more complete inhibition of B7 (and possibly B7-2 and/or B7-3) induction, both the α and β chains can be truncated. See e.g. Griffith et al., *Proc. Natl. Acad. Sci. USA* 85: 4847-4852, (1988), Nabavi et al., *J. Immunol.* 142: 1444-1447, (1989).

Tumor cells can be modified to express an MHC class I molecule by transfection with a nucleic acid encoding an MHC class I α chain protein. For examples of nucleic acids see Hood, L., et al. *Ann. Rev. Immunol.* 1, 529-568 (1983) and Auffray, C. and Strominger, J.L., *Advances in Human Genetics* 15, 197-247 (1987). Optionally, if the tumor cell does not

express β-2 microglobulin, it can also be transfected with a nucleic acid encoding the β-2 microglobulin protein. For examples of nucleic acids see Gussow, D., et al., *J. Immunol.* 139, 3132-3138 (1987) and Parnes, J.R., et al., *Proc. Natl. Acad. Sci. USA* 78, 2253-2257 (1981). As for MHC class II molecules, increasing the number of different MHC class I genes or polymorphic alleles of MHC class I genes expressed in a tumor cell can increase the spectrum of antigenic fragments that the tumor cells can present to T cells.

When a tumor cell is transfected with nucleic acid which encodes more than one molecule, for example a B7-2 and/or B7-3 molecule(s), an MHC class II α chain protein and an MHC class II β chain protein, the transfections can be performed simultaneously or sequentially. If the transfections are performed simultaneously, the molecules can be introduced on the same nucleic acid, so long as the encoded sequences do not exceed a carrying capacity for a particular vector used. Alternatively, the molecules can be encoded by separate nucleic acids. If the transfections are conducted sequentially and tumor cells are selected using a selectable marker, one selectable marker can be used in conjunction with the first introduced nucleic acid while a different selectable marker can be used in conjunction with the next introduced nucleic acid.

The expression of MHC molecules (class I or class II) on the cell surface of a tumor cell can be determined, for example, by immunoflourescence of tumor cells using fluorescently labeled monoclonal antibodies directed against different MHC molecules. Monoclonal antibodies which recognize either non-polymorphic regions of a particular MHC molecule (non-allele specific) or polymorphic regions of a particular MHC molecule (allele-specific) can be used and are known to those skilled in the art.

B. Induction or Increased Expression of MHC Molecules on a Tumor Cell

Another approach to modifying a tumor cell *ex vivo* to express MHC molecules on the surface of a tumor cell is to use an agent which stimulates expression of MHC molecules in order to induce or increase expression of MHC molecules on the tumor cell surface. For example, tumor cells can be contacted with the agent *in vitro* in a culture medium. An agent which stimulates expression of MHC molecules may act, for instance, by increasing transcription of MHC class I and/or class II genes, by increasing translation of MHC class I and/or class II mRNAs or by increasing stability or transport of MHC class I and/or class II proteins to the cell surface. A number of agents have been shown to increase the level of cell-surface expression of MHC class II molecules. See for example Cockfield, S.M. et al., *J. Immunol.* 144, 2967-2974 (1990); Noelle, R.J. et al. *J. Immunol.* 137, 1718-1723 (1986); Mond, J.J., et al., *J. Immunol.* 127, 881-888 (1981); Willman, C.L., et al. *J. Exp. Med.*, 170, 1559-1567 (1989); Celada, A.and Maki, R. *J. Immunol.* 146, 114-120 (1991) and Glimcher, L.H. and Kara, C.J. *Ann. Rev. Immunol.* 10, 13-49 (1992) and references therein. These agents include cytokines, antibodies to other cell surface molecules and phorbol esters. One agent which upregulates MHC class I and class II molecules on a wide variety of cell types is

the cytokine interferon-γ. Thus, for example, tumor cells modified to express B7-2 and/or B7-3 can be further modified to increase expression of MHC molecules by contact with interferon-γ.

Another agent which can be used to induce or increase expression of an MHC molecule on a tumor cell surface is a nucleic acid encoding a transcription factor which upregulates transcription of MHC class I or class II genes. Such a nucleic acid can be transfected into the tumor cell to cause increased transcription of MHC genes, resulting in increased cell-surface levels of MHC proteins. MHC class I and class II genes are regulated by different transcription factors. However, the multiple MHC class I genes are regulated coordinately, as are the multiple MHC class II genes. Therefore, transfection of a tumor cell with a nucleic acid encoding a transcription factor which regulates MHC gene expression may increase expression of several different MHC molecules on the tumor cell surface. Several transcription factors which regulate the expression of MHC genes have been identified, cloned and characterized. For example, see Reith, W. et al., *Genes Dev.* 4, 1528-1540, (1990); Liou, H.-C., et al., *Science* 247, 1581-1584 (1988); Didier, D.K., et al., *Proc. Natl. Acad. Sci. USA* 85, 7322-7326 (1988).

III. Inhibition of Invariant Chain Expression in Tumor Cells

Another embodiment of the invention provides a tumor cell modified to express a T cell costimulatory molecule (e.g., B7-2 and/or B7-3) and in which expression of an MHC class II-associated protein, the invariant chain, is inhibited. Invariant chain expression is inhibited to promote association of endogenously-derived TAA peptides with MHC class II molecules to create an antigen-MHC complex. This complex can trigger an antigen-specific signal in T cells to induce activation of T cells in conjunction with a costimulatory signal. MHC class II molecules have been shown to be capable of presenting endogenously-derived peptides. Nuchtern, J.G., et al. *Nature* 343, 74-76 (1990); Weiss, S. and Bogen, B. *Cell* 767-776 (1991). However, in cells which naturally express MHC class II molecules, the α and β chain proteins are associated with the invariant chain (hereafter Ii) during intracellular transport of the proteins from the endoplasmic reticulum. It is believed that Ii functions in part by preventing the association of endogenously-derived peptides with MHC class II molecules. Elliott, W., et al. *J. Immunol.* 138, 2949-2952 (1987); Stockinger, B., et al. *Cell* 56, 683-689 (1989); Guagliardi, L., et al. *Nature (London)* 343, 133-139 (1990); Bakke, O., et al. *Cell* 63, 707-716 (1990); Lottreau, V., et al. *Nature* 348,600-605 (1990); Peters, J., et al. *Nature* 349, 669-676 (1991); Roche, P., et al.*Nature* 345, 615-618 (1990); Teyton, L., et al. *Nature* 348, 39-44 (1990). Since TAAs are synthesized endogenously in tumor cells, peptides derived from them are likely to be available intracellularly. Accordingly, inhibiting the expression of Ii in tumor cells which express Ii may increase the likelihood that TAA peptides will associate with MHC class II molecules. Consistent with this mechanism, it was shown that supertransfection of an MHC class II^+, Ii^- tumor cell with the Ii gene prevented

stimulation of tumor-specific immunity by the tumor cell. Clements, V.K., et al. *J. Immunol.* 149, 2391-2396 (1992).

Prior to modification, the expression of Ii in a tumor cell can be assessed by detecting the presence or absence of Ii mRNA by Northern blotting or by detecting the presence or absence of Ii protein by immunoprecipitation. A preferred approach for inhibiting expression of Ii is by introducing into the tumor cells a nucleic acid which is antisense to a coding or regulatory region of the Ii gene, which have been previously described. Koch, N., et al., *EMBO J.* 6, 1677-1683, (1987). For example, an oligonucleotide complementary to nucleotides near the translation initiation site of the Ii mRNA can be synthesized. One or more antisense oligonucleotides can be added to media containing tumor cells, typically at a concentration of oligonucleotides of 200 μg/ml. The antisense oligonucleotide is taken up by tumor cells and hybridizes to Ii mRNA to prevent translation. In another embodiment, a recombinant expression vector is used in which a nucleic acid encoding sequences of the Ii gene in an orientation such that mRNA which is antisense to a coding or regulatory region of the Ii gene is produced. Tumor cells transfected with this recombinant expression vector thus contain a continuous source of Ii antisense nucleic acid to prevent production of Ii protein. Alternatively, Ii expression in a tumor cell can be inhibited by treating the tumor cell with an agent which interferes with Ii expression. For example, a pharmaceutical agent which inhibits Ii gene expression, Ii mRNA translation or Ii protein stability or intracellular transport can be used.

IV. Types of Tumor Cells to be Modified

The tumor cells to be modified as described herein include tumor cells which can be transfected or treated by one or more of the approaches encompassed by the present invention to express B7-2 and/or B7-3, alone or in combination with B7. If necessary, the tumor cells can be further modified to express MHC molecules or an inhibitor of Ii expression. A tumor from which tumor cells are obtained can be one that has arisen spontaneously, e.g in a human subject, or may be experimentally derived or induced, e.g. in an animal subject. The tumor cells can be obtained, for example, from a solid tumor of an organ, such as a tumor of the lung, liver, breast, colon, bone etc. Malignancies of solid organs include carcinomas, sarcomas, melanomas and neuroblastomas. The tumor cells can also be obtained from a blood-borne (ie. dispersed) malignancy such as a lymphoma, a myeloma or a leukemia.

The tumor cells to be modified include those that express MHC molecules on their cell surface prior to transfection and those that express no or low levels of MHC class I and/or class II molecules. A minority of normal cell types express MHC class II molecules. It is therefore expected that many tumor cells will not express MHC class II molecules naturally. These tumors can be modified to express B7-2 and/or B7-3 and MHC class II molecules. Several types of tumors have been found to naturally express surface MHC class II molecules, such as melanomas (van Duinen et al., *Cancer Res.* 48, 1019-1025, 1988),

diffuse large cell lymphomas (O'Keane et al., *Cancer* 66, 1147-1153, 1990), squamous cell carcinomas of the head and neck (Mattijssen et al., *Int. J. Cancer* 6, 95-100, 1991) and colorectal carcinomas (Moller et al., *Int. J. Cancer* 6, 155-162, 1991). Tumor cells which naturally express class II molecules can be modified to express B7-2 and/or B7-3, and, in addition, other class II molecules which can increase the spectrum of TAA peptides which can be presented by the tumor cell. Most non-malignant cell types express MHC class I molecules. However, malignant transformation is often accompanied by downregulation of expression of MHC class I molecules on the surface of tumor cells. Csiba, A., et al., *Brit. J. Cancer* 50, 699-709 (1984). Importantly, loss of expression of MHC class I antigens by tumor cells is associated with a greater aggressiveness and/or metastatic potential of the tumor cells. Schrier, P.I., et al. *Nature* 305, 771-775 (1983); Holden, C.A., et al. *J. Am. Acad. Dermatol.* 9., 867-871 (1983); Baniyash, M., et al. *J. Immunol.* 129, 1318-1323 (1982). Types of tumors in which MHC class I expression has been shown to be inhibited include melanomas, colorectal carcinomas and squamous cell carcinomas. van Duinen et al., *Cancer Res.* 48, 1019-1025, (1988); Moller et al., *Int. J. Cancer* 6, 155-162, (1991); Csiba, A., et al., *Brit. J. Cancer* 50, 699-709 (1984); Holden, C.A., et al. *J. Am. Acad. Dermatol.* 9., 867-871 (1983). A tumor cell which fails to express class I molecules or which expresses only low levels of MHC class I molecules can be modified by one or more of the techniques described herein to induce or increase expression of MHC class I molecules on the tumor cell surface to enhance tumor cell immunogenicity.

V. Modification of Tumor Cells *In Vivo*

Another aspect of the invention provides methods for increasing the immunogenicity of a tumor cell by modification of the tumor cell *in vivo* to express B7-2 and/or B7-3 to trigger a costimulatory signal in T cells. In addition, tumor cells can be further modified *in vivo* to express MHC molecules to trigger a primary, antigen-specific, signal in T cells. Tumor cells can be modified *in vivo* by introducing a nucleic acid encoding B7-2 and/or B7-3 into the tumor cells in a form suitable for expression of the costimulatory molecule(s) on the surface of the tumor cells. Likewise, nucleic acids encoding MHC class I or class II molecules or an antisense sequence of the Ii gene can be introduced into tumor cells *in vivo*. In one embodiment, a recombinant expression vector is used to deliver nucleic acid encoding B7-2 and/or B7-3 to tumor cells *in vivo* as a form of gene therapy. Vectors useful for *in vivo* gene therapy have been previously described and include retroviral vectors, adenoviral vectors and adeno-associated viral vectors. See e.g. Rosenfeld, M.A., *Cell* 68, 143-155 (1992); Anderson, W.F., *Science* 226, 401-409 (1984); Friedman, T., *Science* 244, 1275-1281 (1989). Alternatively, nucleic acid can be delivered to tumor cells *in vivo* by direct injection of naked nucleic acid into tumor cells. See e.g. Acsadi, G., et al., *Nature* 332, 815-818 (1991). A delivery apparatus is commercially available (BioRad). Optionally, to be suitable for injection, the nucleic acid can be complexed with a carrier such as a liposome. Nucleic

acid encoding an MHC class I molecule complexed with a liposome has been directly injected into tumors of melanoma patients. Hoffman, M., *Science* 256, 305-309 (1992).

Tumor cells can also be modified *in vivo* by use of an agent which induces or increases expression of B7-2 and/or B7-3 (and, if necessary, MHC molecules) as described herein. The agent may be administered systemically, e.g. by intravenous injection, or, preferably, locally to the tumor cells.

VI. The Effector Phase of the Anti-Tumor T Cell-Mediated Immune Response

The modified tumor cells of the invention are useful for stimulating an anti-tumor T cell-mediated immune response by triggering an antigen-specific signal and a costimulatory signal in tumor-specific T cells. Following this inductive, or afferent, phase of an immune response, effector populations of T cells are generated. These effector T cell populations can include both CD4+ T cells and CD8+ T cell. The effector populations are responsible for elimination of tumors cell, by, for example, cytolysis of the tumor cells. Once T cells are activated, expression of a costimulatory molecule is not required on a target cell for recognition of the target cell by effector T cells or for the effector functions of the T cells. Harding, F.A. and Allison, J.P. *J. Exp. Med.* 177, 1791-1796 (1993). Therefore, the anti-tumor T cell-mediated immune response induced by the modified tumor cells of the invention is effective against both the modified tumor cells and unmodified tumor cells which do not express a costimulatory molecule.

Additionally, the density and/or type of MHC molecules on the cell surface required for the afferent and efferent phases of a T cell-mediated immune response can differ. Fewer MHC molecules, or only certain types of MHC molecules (e.g. MHC class I but not MHC class II) may be needed on a tumor cell for recognition by effector T cells than is needed for the initial activation of T cells. Therefore, tumor cells which naturally express low amounts of MHC molecules but are modified to express increased amounts of MHC molecules can induce a T cell-mediated immune response which is effective against the unmodified tumor cells. Alternatively, tumor cells which naturally express MHC class I molecules but not MHC class II molecules which are then modified to express MHC class II molecules can induce a T cell-mediated immune response which includes effector T cell populations which can eliminate the parental MHC class I+, class II– tumor cells.

VII. Therapeutic Compositions of Tumor Cells

Another aspect of the invention is a composition of modified tumor cells in a biologically compatible form suitable for pharmaceutical administration to a subject *in vivo*. This composition comprises an amount of modified tumor cells and a physiologically acceptable carrier. The amount of modified tumor cells is selected to be therapeutically effective. The term "biologically compatible form suitable for pharmaceutical administration *in vivo*" means that any toxic effects of the tumor cells are outweighed by the therapeutic

effects of the tumor cells. A "physiologically acceptable carrier" is one which is biologically compatible with the subject. Examples of acceptable carriers include saline and aqueous buffer solutions. In all cases, the compositions must be sterile and must be fluid to the extent that easy syringability exists. The term "subject" is intended to include living organisms in which tumors can arise or be experimentally induced. Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof.

Administration of the therapeutic compositions of the present invention can be carried out using known procedures, at dosages and for periods of time effective to achieve the desired result. For example, a therapeutically effective dose of modified tumor cells may vary according to such factors as age, sex and weight of the individual, the type of tumor cell and degree of tumor burden, and the immunological competency of the subject. Dosage regimens may be adjusted to provide optimum therapeutic responses. For instance, a single dose of modified tumor cells may be administered or several doses may be administered over time. Administration may be by injection, including intravenous, intramuscular, intraperitoneal and subcutaneous injections.

VIII. Activation of Tumor-specific T Lymphocytes *In Vitro*

Another approach to inducing or enhancing an anti-tumor T cell-mediated immune response by triggering a costimulatory signal in T cells is to obtain T lymphocytes from a tumor-bearing subject and activate them *in vitro* by stimulating them with tumor cells and a stimulatory form of B7-2 and/or B7-3. T cells can be obtained from a subject, for example, from peripheral blood. Peripheral blood can be further fractionated to remove red blood cells and enrich for or isolate T lymophocytes or T lymphocyte subpopulations. T cells can be activated *in vitro* by culturing the T cells with tumor cells obtained from the subject (e.g. from a biopsy or from peripheral blood in the case of blood-borne malignancies) together with a stimulatory form of B7-2 and/or B7-3 or, alternatively, by exposure to a modified tumor cell as described herein. The term "stimulatory form" means that the costimulatory molecule is capable of crosslinking its receptor on a T cell and triggering a costimulatory signal in T cells. The stimulatory form of the costimulatory molecule can be, for example, a soluble multivalent molecule or an immobilized form of the costimulatory molecule, for instance coupled to a solid support. Fragments, mutants or variants (e.g. fusion proteins) of B7-2 and/or B7-3 which retain the ability to trigger a costimulatory signal in T cells can also be used. In a preferred embodiment, a soluble extracellular portion of B7-2 and/or B7-3 is used to provide costimulation to the T cells. Following culturing of the T cells *in vitro* with tumor cells and B7-2 and/or B7-3, or a modified tumor cell, to activate tumor-specific T cells, the T cells can be administered to the subject, for example by intravenous injection.

IX. Therapeutic Uses of Modified Tumor Cells

The modified tumor cells of the present invention can be used to increase tumor immunogenicity, and therefore can be used therapeutically for inducing or enhancing T lymphocyte-mediated anti-tumor immunity in a subject with a tumor or at risk of developing a tumor. A method for treating a subject with a tumor involves obtaining tumor cells from the subject, modifying the tumor cells *ex vivo* to express a T cell costimulatory molecule, for example by transfecting them with an appropriate nucleic acid, and administering a therapeutically effective dose of the modified tumor cells to the subject. Appropriate nucleic acids to be introduced into a tumor cell include nucleic acids encoding B7-2 and/or B7-3, alone or together with nucleic acids encoding B7, MHC molecules (class I or class II) or Ii antisense sequences as described herein. Alternatively, after tumor cells are obtained from a subject, they can be modified *ex vivo* using an agent which induces or increases expression of B7-2 and/or B7-3 (and possibly also using agent(s) which induce or increase B7 or MHC molecules).

Tumor cells can be obtained from a subject by, for example, surgical removal of tumor cells, e.g. a biopsy of the tumor, or from a blood sample from the subject in cases of blood-borne malignancies. In the case of an experimentally induced tumor, the cells used to induce the tumor can be used, e.g. cells of a tumor cell line. Samples of solid tumors may be treated prior to modification to produce a single-cell suspension of tumor cells for maximal efficiency of transfection. Possible treatments include manual dispersion of cells or enzymatic digestion of connective tissue fibers, e.g. by collagenase.

Tumor cells can be transfected immediately after being obtained from the subject or can be cultured *in vitro* prior to transfection to allow for further characterization of the tumor cells (e.g. determination of the expression of cell surface molecules). The nucleic acids chosen for transfection can be determined following characterization of the proteins expressed by the tumor cell. For instance, expression of MHC proteins on the cell surface of the tumor cells and/or expression of the Ii protein in the tumor cell can be assessed. Tumors which express no, or limited amounts of or types of MHC molecules (class I or class II) can be transfected with nucleic acids encoding MHC proteins; tumors which express Ii protein can be transfected with Ii antisense sequences. If necessary, following transfection, tumor cells can be screened for introduction of the nucleic acid by using a selectable marker (e.g. drug resistance) which is introduced into the tumor cells together with the nucleic acid of interest.

Prior to administration to the subject, the modified tumor cells can be treated to render them incapable of further proliferation in the subject, thereby preventing any possible outgrowth of the modified tumor cells. Possible treatments include irradiation or mitomycin C treatment, which abrogate the proliferative capacity of the tumor cells while maintaining the ability of the tumor cells to trigger antigen-specific and costimulatory signals in T cells and thus to stimulate an immune response.

The modified tumor cells can be administered to the subject by injection of the tumor cells into the subject. The route of injection can be, for example, intravenous, intramuscular, intraperitoneal or subcutaneous. Administration of the modified tumor cells at the site of the original tumor may be beneficial for inducing local T cell-mediated immune responses against the original tumor. Administration of the modified tumor cells in a disseminated manner, e.g. by intravenous injection, may provide systemic anti-tumor immunity and, furthermore, may protect against metastatic spread of tumor cells from the original site. The modified tumor cells can be administered to a subject prior to or in conjunction with other forms of therapy or can be administered after other treatments such as chemotherapy or surgical intervention.

Additionally, more than one type of modified tumor cell can be administered to a subject. For example, an effective T cell response may require exposure of the T cell to more than one type of costimulatory molecule. Furthermore, the temporal sequence of exposure of the T cell to different costimulatory mocules may be important for generating an effective response. For example, it is known that upon activation, a B cell expresses B7-2 early in its response (about 24 hours after stimulation). Subsequently, B7 and B7-3 are expressed by the B cell (about 48-72 hours after stimulation). Thus, a T cell may require exposure to B7-2 early in the induction of an immune response by exposure to B7 and/or B7-3 in the immune response. Accordingly, different types of modified tumor cells can be administered at different times to a subject to generate an effective immune response against the tumor cells. For example, tumor cells modified to express B7-2 can be administered to a subject. Following this administration, a tumor cell from the same tumor but modified to express B7-3 (alone or in conjunction with B7) can be administered to the subject.

Another method for treating a subject with a tumor is to modify tumor cells *in vivo* to express B7-2 and/or B7-3, alone or in conjunction with B7, MHC molecules and/or an inhibitor of Ii expression. This method can involve modifying tumor cells *in vivo* by providing nucleic acid encoding the protein(s) to be expressed using vectors and delivery methods effective for *in vivo* gene therapy as described in a previous section herein. Alternatively, one or more agents which induce or increase expression of B7-2 and/or B7-3, and possibly B7 or MHC molecules, can be administered to a subject with a tumor.

The modified tumor cells of the current invention may also be used in a method for preventing or treating metastatic spread of a tumor or preventing or treating recurrence of a tumor. As demonstrated in detail in one of the following examples, anti-tumor immunity induced by B7-expressing tumor cells is effective against subsequent challenge by tumor cells, regardless of whether the tumor cells of the re-exposure express B7 or not. Thus, administration of modified tumor cells or modification of tumor cells *in vivo* as described herein can provide tumor immunity against cells of the original, unmodified tumor as well as metastases of the original tumor or possible regrowth of the original tumor.

The current invention also provides a composition and a method for specifically inducing an anti-tumor response in $CD4^+$ T cells. $CD4^+$ T cells are activated by antigen in conjunction with MHC class II molecules. Association of peptidic fragments of TAAs with MHC class II molecules results in recognition of these antigenic peptides by $CD4^+$ T cells. Providing a subject with tumor cells which have been modified to express MHC class II molecules along with B7-2 and/or B7-3, or modified *in vivo* to express MHC class II molecules along with B7-2 and/or B7-3, can be useful for directing tumor antigen presentation to the MHC class II pathway and thereby result in antigen recognition by and activation of $CD4^+$ T cells specific for the tumor cells. As explained in detail in an example to follow, depletion of either $CD4^+$ or $CD8^+$ T cells *in vivo*, by administration of anti-CD4 or anti-CD8 antibodies, can be used to demonstrate that specific anti-tumor immunity is mediated by a particular (e.g. $CD4^+$) T cell subpopulation.

As demonstrated in Example 2, subjects initially exposed to modified tumor cells develop an anti-tumor specific T cell response which is effective against subsequent exposure to unmodified tumor cells. Thus the subject develops anti-tumor specific immunity. The generalized use of modified tumor cells of the invention from one human subject as an immunogen to induce anti-tumor immunity in another human subject is prohibited by histocompatibility differences between unrelated humans. However, use of modified tumor cells from one individual to induce anti-tumor immunity in another individual to protect against possible future occurrence of a tumor may be useful in cases of familial malignancies. In this situation, the tumor-bearing donor of tumor cells to be modified is closely related to the (non-tumor bearing) recipient of the modified tumor cells and therefore the donor and recipient share MHC antigens. A strong hereditary component has been identified for certain types of malignancies, for example certain breast and colon cancers. In families with a known susceptibility to a particular malignancy and in which one individual presently has a tumor, tumor cells from that individual could be modified to express B7-2 and/or B7-3 and administered to susceptible, histocompatible family members to induce an anti-tumor response in the recipient against the type of tumor to which the family is susceptible. This anti-tumor response could provide protective immunity to subsequent development of a tumor in the immunized recipient.

X. Tumor-Specific T Cell Tolerance

In the case of an experimentally induced tumor, such as described in Examples 1 to 3, a subject (e.g. a mouse) can be exposed to the modified tumor cells of the invention before being challenged with unmodified tumor cells. Thus, the subject is initially exposed to TAA peptides on tumor cells together with B7-2 and/or B7-3, which activates TAA-specific T cells. The activated T cells are then effective against subsequent challenge with unmodified tumor cells. In the case of a spontaneously arising tumor, as is the case with human subjects, the subject's immune system will be exposed to unmodified tumor cells before exposure to

the modified tumor cells of the invention. Thus the subject is initially exposed to TAA peptides on tumor cells in the absence of a costimulatory signal. This situation is likely to induce TAA-specific T cell tolerance in those T cells which are exposed to and are in contact with the unmodified tumor cells. Secondary exposure of the subject to modified tumor cells which can trigger a costimulatory signal may not be sufficient to overcome tolerance in TAA-specific T cells which were anergized by primary exposure to the tumor. Use of modified tumor cells to induce anti-tumor immunity in a subject already exposed to unmodified tumor cells may therefore be most effective in early diagnosed patients with small tumor burdens, for instance a small localized tumor which has not metastasized. In this situation, the tumor cells are confined to a limited area of the body and thus only a portion of the T cell repertoire may be exposed to tumor antigens and become anergized. Administration of modified tumor cells in a systemic manner, for instance after surgical removal of the localized tumor and modification of isolated tumor cells, may expose non-anergized T cells to tumor antigens together with B7-2 and/or B7-3, thereby inducing an anti-tumor response in the non-anergized T cells. The anti-tumor response may be effective against possible regrowth of the tumor or against micrometastases of the original tumor which may not have been detected. To overcome widespread peripheral T cell tolerance to tumor cells in a subject, additional signals, such as a cytokine, may need to be provided to the subject together with the modified tumor cells. A cytokine which functions as a T cell growth factor, such as IL-2, could be provided to the subject together with the modified tumor cells. IL-2 has been shown to be capable of restoring the alloantigen-specific responses of previously anergized T cells in an *in vitro* system when exogenous IL-2 is added at the time of secondary alloantigenic stimulation. Tan, P., et al. *J. Exp. Med.* 177, 165-173 (1993).

Another approach to generating an anti-tumor T cell response in a subject despite tolerance of the subject's T cells to the tumor is to stimulate an anti-tumor response in T cells from another subject who has not been exposed to the tumor (referred to as a naive donor) and transfer the stimulated T cells from the naive donor back into the tumor-bearing subject so that the transferred T cells can mount an immune response against the tumor cells. An anti-tumor response is induced in the T cells from the naive donor by stimulating the T cells *in vitro* with the modified tumor cells of the invention. Such an adoptive transfer approach is generally prohibited in outbred populations because of histocompatibity differences between the transferred T cells and the tumor-bearing recipient. However, advances in allogeneic bone marrow transplantation can be applied to this situation to allow for acceptance by the recipient of the adoptively transferred cells and prevention of graft versus host disease. First, a tumor-bearing subject (referred to as the host) is prepared for and receives an allogeneic bone marrow transplant from a naive donor by a known procedure. Preparation of the host involves whole body irradiation, which destroys the host's immune system, including T cells tolerized to the tumor, as well as the tumor cells themselves. Bone marrow transplantation is accompanied by treatment(s) to prevent graft versus host disease such as depletion of mature

T cells from the bone marrow graft, treatment of the host with immunosuppressive drugs or treatment of the host with an agent, such as CTLA4Ig, to induce donor T cell tolerance to host tissues. Next, to provide anti-tumor specific T cells to the host which can respond against residual tumor cells in the host or regrowth or metastases of the original tumor in the host, T cells from the naive donor are stimulated *in vitro* with tumor cells from the host which have been modified, as described herein, to express B7-2 and/or B7-3. Thus, the donor T cells are initially exposed to tumor cells together with a costimulatory signal and therefore are activated to respond to the tumor cells. These activated anti-tumor specific T cells are then transferred to the host where they are reactive against unmodified tumor cells. Since the host has been reconstituted with the donor's immune system, the host will not reject the transferred T cells and, additionally, the treatment of the host to prevent graft versus host disease will prevent reactivity of the transferred T cells with normal host tissues.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated by reference.

In Examples 1-3, mouse sarcoma cells were modified to express the T cell costimulatory molecule B7. The following methodology was used in Examples 1 to 3.

Methods and Materials

A. Cells

SaI tumor cells were maintained as described (Ostrand-Rosenberg, S., et al., *J. Immunol.* 144, 4068-4071 (1990)).

B. Antibodies

The monoclonal antibody (mAb) 10-3.6, specific for I-A^k (Oi, V., et al. *Curr. Top. Microbiol. Immunol.* 81, 115-120 (1978)), was prepared and used as described. Ostrand-Rosenberg, S., et al., *J. Immunol.* 144: 4068-4071 (1990). The B7-specific mAb 1G10 is a rat IgG2a mAb and was used as described (Nabavi, N., et al. *Nature* 360, 266-268 (1992)). mAbs specific for $CD4^+$ [GK1.5 (Wilde, D.B., et al. *J. Immunol.* 131, 2178-2183 (1983))] and $CD8^+$ [2.43 (Sarmiento, M., et al. *J. Immunol.* 125, 2665-2672 (1980))] were used as ascites fluid.

C. Transfections

Mouse SaI sarcoma cells were transfected as described in Ostrand-Rosenberg, S., et al., *J. Immunol.* 144, 4068-4071 (1990). SaI cells (2×10^6) were transfected by the calcium phosphate method (Wigler et al., *Proc. Natl. Acad. Sci. USA*, 76, 1373 (1979)). SaI cells were transfected with wild-type $A\alpha^k$ and $A\beta^k$ MHC class II cDNAs (Ostrand-Rosenberg, S., et al., *J. Immunol.* 144, 4068-4071 (1990)), $A\alpha^k$ and $A\beta^k$ cDNAs truncated for their C-terminal 12 and 10 amino acids, respectively (Nabavi, N., et al. *J. Immunol.* 142, 1444-1447 (1989)), and/or B7 gene (Freeman, G.J., et al. *J. Exp. Med.* 174, 625-631 (1991)). For

transfection, the murine B7 cDNA was subcloned into the eukaryotic expression vector dCDNAI (Invitrogen, San Diego, CA). Class II transfectants were cotransfected with pSV2neo plasmid and selected for resistance to G418 (400 μg/ml). B7 transfectants were cotransfected with pSV2hph plasmid and selected for hygromycin-resistance (400 μg/ml). All transfectants were cloned twice by limiting dilution, except SaI/B7 transfectants, which were uncloned, and maintained in drug. Double transfectants were maintained in G418 plus hygromycin. The numbers after each transfectant are the clone designation.

D. Immunofluorescence

Indirect immunofluorescence was performed as described (Ostrand-Rosenberg. S., et al., *J. Immunol.* 144, 4068-4071 (1990)), and samples were analyzed on an Epics C flow cytometer.

E. Tumor Challenges

For primary tumor challenges, autologous A/J mice were challenged intraperitoneally (i.p.) with the indicated number of tumor cells. Inoculated mice were checked three times per week for tumor growth. Mean survival times of mice dying from their tumor ranged from 13 to 28 days after inoculation. Mice were considered to have died from their tumor if they contained a large volume of ascites fluid and tumor cells (≥5 ml) at the time of death. Mice were considered tumor-resistant if they were tumor-free for at least 60 days after tumor challenge (range, 60-120 days). Tumor cells were monitored by indirect immunofluorescence for I-A^k and B7 expression prior to tumor-cell inoculation. For the experiments of Table 2, autologous A/J mice were immunized i.p. with a single inoculum of the indicated number of live tumor cells and challenged i.p. with the indicated number of wild-type SaI cells 42 days after immunization. Mice were evaluated for tumor resistance or susceptibility using the same criteria as for primary tumor challenge.

F. *In vivo* T cell Depletions

A/J mice were depleted of $CD4^+$ or $CD8^+$ T cells by i.p. inoculation with 100 μl of ascites fluid of mAb GK1.5 ($CD4^+$ specific; Wilde, D.B., et al., *J. Immunol.* 131, 2178-2183 (1983)) or mAb 2.43 ($CD8^+$ specific; Sarmiento, M., et al., *J. Immunol.* 125, 2665-2672 (1980)) on days -6,-3, and -1 prior to tumor challenge, and every third day after tumor challenge as described (Ghobrial, M., et al. *Clin. Immunol. Immunopathol.* 52, 486-506 (1989)) until the mice died or day 28, whichever came first. Presence or absence of tumor was assessed up to day 28. Previous studies have established that A/J mice with large tumors at day 28 after injection will progress to death. This time point was, therefore, chosen to assess tumor susceptibility for the *in vivo* depletion experiments. One mouse per group was sacrificed on day 28, and its spleen was assayed by immunofluorescence to ascertain depletion of the relevant T cell population.

Example 1: Coexpression of B7 Restores Tumor Immunogenicity

A mouse sarcoma cell line SaI was used in each of the examples. The mouse SaI sarcoma is an ascites-adapted class I^+ class II^- tumor of A/J (H-$2K^kA^k$ D^d) mice. The wild-type tumor is lethal in autologous A/J mice when administered i.p. It has previously been shown that SaI cells transfected with, and expressing, syngeneic MHC class II genes ($A\alpha^k$ and $A\beta^k$ genes; SaI/A^k cells) are immunologically rejected by the autologous host, and immunization with live SaI/A^k cells protects mice against subsequent challenges with wild-type class II^- SaI cells (Ostrand-Rosenberg, S., et al., *J. Immunol.* 144, 4068-4071 (1990)). Adoptive transfer (Cole, G., et al. *Cell. Immunol.* 134, 480-490 (1991)) and lymphocyte depletion studies (E. Lamousse-Smith and S.O.-R., unpublished data) demonstrate that SaI and SaI/A^k rejection is dependent on $CD4^+$ lymphocytes. SaI cells expressing class II molecules with truncated cytoplasmic domains (SaI/A^ktr cells), however, are as lethal as wild-type class II^- SaI cells, suggesting that the cytoplasmic region of the class II heterodimer is required to induce protective immunity (Ostrand-Rosenberg, S., et al. *J. Immunol.* 147, 2419-2422 (1991)).

Up-regulation of the B7 activation molecule on APCs is triggered by intracellular signals transmitted by the cytoplasmic domain of the class II heterodimer, after presentation of antigen to $CD4^+$ T helper cells (Nabavi, N., et al., *Nature* 360, 266-268 (1992)). Inasmuch as B7 expression is normally up-regulated *in vivo* on SaI cells expressing full-length class II molecules (S.B. and S.O.-R., unpublished data), it may be that SaI/A^ktr cells do not stimulate protective immunity because they do not transmit a costimulatory signal.

To test whether B7 expression can compensate for the absence of the class II cytoplasmic domain, SaI/A^ktr cells were supertransfected with a plasmid containing a cDNA encoding murine B7 under the control of the cytomegalovirus promoter and screened for I-A^k and B7 expression by indirect immunofluorescrence. Wild-type SaI cells do not express either I-A^k or B7 (Fig. 1 *a* and *b*), whereas SaI cells transfected with $A\alpha^k$ and $A\beta^k$ genes (SaI/A^k cells) express I-A^k (Fig. 1 *d* and *f*) and do not express B7 (Fig. 1 *c* and *e*). SaI cells transfected with truncated class II genes plus the B7 gene (SaI/A^ktr/B7 cells) express I-A^k and B7 molecules (Fig. 1 *g* and *h*). All cells express uniform levels of MHC class I molecules (K^k and D^d) comparable to the level of I-A^k in Fig. 1*h*.

Antigen-presenting activity of the transfectants was tested by determining their immunogenicity and lethality in autologous A/J mice. As shown in Table 1, wild-type SaI cells administered i.p. at doses as low as 10^4 cells are lethal in 88-100% of mice inoculated within 13-28 days after challenge, whereas 100 times as many SaI/A^k cells are uniformly rejected. Challenges with similar quantities of SaI/A^ktr cells are also lethal; however, SaI/A^ktr cells that coexpress B7 (SaI/A^ktr/B7 clone-1 and clone-3) are uniformly rejected. A/J mice challenged with SaI/A^ktr cells transfected with the B7 construct, but not expressing detectable amounts of B7 antigen (SaI/A^ktr/hph cells), are as lethal as SaI/A^ktr cells.

demonstrating that reversal of the malignant phenotype in SaI/A^ktr/B7 cells is due to expression of B7. SaI cells transfected with the B7 gene and not coexpressing truncated class II molecules (SaI/B7 cells, uncloned) are also as lethal as wild-type SaI cells, indicating the B7 expression without truncated class II molecules does not stimulate immunity. To ascertain that rejection of SaI/A^k and SaI/A^ktr/B7 cells is immunologically mediated, sublethally irradiated (900 rads; 1 rad = 0.01 Gy) A/J mice were challenged i.p. with these cells. In all cases, irradiated mice died from the tumor. Thus, immunogenicity and host rejection of the MHC class II^+ tumor cells are dependent on an intact class II molecule and that coexpression of B7 can bypass the requirement of the class II intracellular domain.

Table 1: Tumorigenicity of B7 and MHC class II-transfected SaI tumor cells

Challenge tumor	Expression I-A^k	Expression B7	Tumor dose, no. of cells	Mice dead/mice tested, no./no.
SaI	-	-	1×10^6	9/10
	-	-	1×10^5	8/10
	-	-	1×10^4	7/8
SaI/A^k 19.6.4	A^k	-	1×10^6	0/12
	A^k	-	5×10^5	0/5
	A^k	-	1×10^5	0/5
SaI/A^ktr 6.11.8	A^ktr	-	1×10^6	12/12
	A^ktr	-	5×10^5	5/5
	A^ktr	-	1×10^5	5/10
SaI/A^ktr/B7-Clone 1	A^ktr	B7	1×10^6	0/4
SaI/A^ktr/B7-Clone 3	A^ktr	B7	1×10^6	0/5
	A^ktr	B7	4×10^5	0/5
	A^ktr	B7	1×10^5	0/5
SaI/A^ktr/hph	A^ktr	-	1×10^6	5/5
SaI/B7	-	B7	1×10^6	5/5

Example 2: Immunization with B7-Transfected Sarcoma Cells Protects Against Later Challenges of Wild-Type $B7^-$ Sarcoma

Activation of at least some T cells is thought to be dependent on coexpression of B7. However, once the T cells are activated, B7 expression is not required on the target T cell for recognition by effector T cells. The ability of three SaI/A^ktr/B7 clones (B7-clone 3, B7-clone 1, and B7-2B5.F2) to immunize A/J mice against subsequent challenges of wild-type class II^- $B7^-$ SaI cells (Table 2) was determined. A/J mice were immunized with live

SaI/A^{k}tr/B7 transfectants and 42 days later challenged with wild-type SaI tumor cells. Ninety-seven percent of mice immunized with SaI/A^{k}tr/B7 transfectants were immune to ≥ 10^6 wild-type B7$^-$ class II$^-$ SaI cells, an immunity that is comparable to that induced by immunization with SaI cells expressing full-length class II molecules. SaI/A^{k}tr/B7 cells, therefore, stimulate a potent response with long-term immunological memory against high-dose challenges of malignant tumor cells. B7 expression is, therefore, critical for the stimulation of SaI-specific effector cells; however, its expression is not needed on the tumor targets once the appropriate effector T cell populations have been generated.

Table 2. Autologous A/J mice immunized with SaI/A^{k}tr/B7 cells are immune to challenges of wild-type SaI tumor

Immunization	No. of immunizing cells	SaI challenge dose no. of cells	Mice dead/ mice tested no./no.
None	-	1×10^6	5/5
SaI/A^k 19.6,4	1×10^5 or 10^6	1×10^6	0/5
	1×10^6	6×10^6	0/5
SaI/A^{k}tr/B7-clone 3	1×10^6	6×10^6	0/5
	1×10^6	1×10^6	0/5
	4×10^5	1×10^6	0/5
	1×10^5	5×10^6	0/5
SaI/A^{k}tr/B7-clone 1	5×10^5	3×10^6	0/3
	2×10^5	1×10^6	0/2
	5×10^4	5×10^6	0/3
SaI/A^{k}tr/B7-2B5.E2	1×10^5	2×10^6	0/2
	5×10^4	2×10^6	1/7

Example 3: Immunization with B7-Transfected Tumor Cells Stimulates Tumor-Specific CD4$^\pm$ Lymphocytes

To ascertain that B7 is functioning through a T cell pathway in tumor rejection, we have *in vivo*-depleted A/J mice for CD4$^+$ or CD8$^+$ T cells and challenged them i.p with SaI/A^k or SaIA^{k}tr/B7 cells. As shown in Table 3, *in vivo* depletion of CD4$^+$ T cells results in host susceptibility to both SaI/A^k and SaI/A^{k}tr/B7 tumors, indicating that CD4$^+$ T cells are critical for tumor rejection, whereas depletion of CD8$^+$ T cells does not affect SaI/A^{k}tr/B7 tumor rejection. Although immunofluorescence analysis of splenocytes of

$CD8^+$-depleted mice demonstrates the absence of $CD8^+$ T cells, it is possible that the depleted mice contain small quantitites of $CD8^+$ cells that are below our level of detection. These data therefore demonstrate that $CD4^+$ T cells are required for tumor rejection but do not eliminate a possible corequirement for $CD8^+$ T cells.

Table 3. Tumor susceptibility of A/J mice *in vivo*-depleted for $CD4^+$ or $CD8^+$ T cells

Tumor challenge	Host T cell depletion	No. mice with tumor/ total no. mice challenged
SaI/A^k	$CD4^+$	3/5
SaI/A^ktr/B7-clone 3	$CD4^+$	5/5
	$CD8^+$	0/5

Previous adoptive transfer experiments (Cole, G., et al. *Cell. Immunol.* 134, 480-490 (1991)) have demonstrated that both $CD4^+$ and $CD8^+$ T cells are required for rejection of class II wild-type SaI cells. Inasmuch as rejection of SaI/A^k and SaI/A^ktr/B7 cells appears to require only $CD4^+$ T cells, it is likely that immunization with class II^+ transfectants stimulates both $CD4^+$ and $CD8^+$ effector T cells; however, only the $CD8^+$ effectors are required for rejection of class I^+ II^- tumor targets. Costimulation by B7, therefore, enhances immunity by stimulating tumor-specific $CD4^+$ helper and cytotoxic lymphocytes.

Example 4: Determination of the Effect of Modified Tumor Cells in Subjects Previously Exposed to Unmodified Tumor Cells

In the previous examples, mice were immunized with modified tumor cells to which they had not been previously exposed. In the case of treating a subject with a pre-existing tumor, the subject will be exposed to unmodified tumor cells for a period of time before exposure to modified tumor cells, and therefore the subject may become tolerized to the unmodified tumor cells.

To determine whether the modified tumor cells of the invention which express B7-2 and/or B7-3 are effective in overcoming tolerance and inducing an anti-tumor T cell response in a subject, mice are inoculated with increasing amounts of wild-type SaI tumor cells which have been irradiated with 10,000 rads. Doses of tumor cells in the range of $1x10^4$ to $1x10^6$ cells can be inoculated. Tumor cells irradiated in this way survive for up to two months in the recipient mice, sufficient time for tolerance to the tumor cells to be induced in the mice. After two months exposure to the wild-type tumor cells, mice are injected simultaneously with wild-type tumor cells into the flank of one hind leg and with tumor cells modified to express B7-2 and/or B7-3 into the flank of the opposite hind leg. As a control, mice are injected with wild-type tumor cells into both flanks. Tumor cell doses in the range of $1x10^4$

to $1x10^6$ cells are used for challenges. Tumor growth is assessed by measuring the size of a tumor which grows at the site of injection. The ability of tumor cells modified to express B7-2 and/or B7-3 to induce anti-tumor immunity, and therefore overcome any possible tolerance to the tumor cells in the mice, is determined by the ability of the modified tumor cells injected into one flank to prevent growth of wild-type tumor cells in the opposite flank, as compared to when wild-type tumor cells are injected into both flanks.

Alternatively, the ability of tumor cells modified to express B7-2 and/or B7-3 to overcome potential tolerance to unmodified tumor cells is assessed by an adoptive transfer experiment. A mouse is injected intraperitoneally with a low dose, e.g. $1x10^4$ cells, of wild-type SaI cells and the tumor cells are allowed to grow for three weeks, at which time the mouse is sacrificed and spleen cells from the mouse are harvested. These spleen cells are injected intraperitoneally into a recipient, syngeneic mouse which has been lethally irradiated to destroy its endogenous immune system. The adoptively transferred spleen cells reconstitute the recipient mouse with an immune system which has been previously exposed to wild-type tumor cells. Following spleen cell transfer, the recipient mouse is then challenged with wild-type tumor cells injected into the flank of one hind leg and with tumor cells modified to express B7-2 and/or B7-3 injected into the flank of the opposite hind leg. Tumor cell doses in the range of $1x10^4$ to $1x10^6$ cells are used for challenges. The ability of the modified tumor cells to induce anti-tumor immunity is determined by the ability of the modified tumor cells injected into one flank to prevent the growth of wild-type tumor cells injected into the opposite flank.

Example 5: Regression of Implanted Tumor Cells Transfected to Express B7-2

In this example, untransfected or B7-2 transfected J558 plasmacytoma cells were used in tumor regression studies to examine the effect of expression of B7-2 on the surface of tumor cells on the growth of the tumor cells when transplanted into animals.

J558 plasmacytoma cells (obtained from the American Type Culture Collection, Rockville, MD; # TIB 6) were transfected with an expression vector containing cDNA encoding either mouse B7-2 (pAWNE03) or B7-1 (pNRDSH or pAWNE03) and a neomycin-resistance gene. Stable transfectants were selected based upon their neomycin resistance and cell surface expression of B7-2 or B7-1 on the tumor cells was confirmed by FACS analysis using either an anti-B7-2 or anti-B7-1 antibody.

Syngeneic Balb/c mice, in groups of 5-10 mice/set, were used in experiments designed to determine whether cell-surface expression of B7-2 on tumor cells would result in regression of the implanted tumor cells. Untransfected and transfected J558 cells were cultured *in vitro*, collected, washed and resuspended in Hank's buffered salt solution (GIBCO, Grand Island, New York) at a concentration of 10^8 cells/ml. A patch of skin on the right flank of each mouse was removed of hair with a depilatory and, 24 hours later, 5×10^6 tumor cells/mouse were implanted intradermally or subdermally. Measurements of tumor

volume (by linear measurements in three perpendicular directions) were made every two to three days using calipers and a ruler. A typical experiment lasted 18-21 days, after which time the tumor size exceeded 10 % of the body mass of mice transplanted with untransfected, control J558 cells. As shown in Figure 2, J558 cells transfected to express B7-2 on their surface were rejected by the mice. No tumor growth was observed even after three weeks. Similar results were observed with J558 cells transfected to express B7-1 on their surface. In contrast, the untransfected (wild-type) J558 cells produced massive tumors in as little as 12 days, requiring the animal to be euthanized. This example demonstrates that cell-surface expression of B7-2 on tumor cells, such as by transfection of the tumor cells with a B7-2 cDNA, induces an anti-tumor response in naive animals that is sufficient to cause rejection of the tumor cells.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Freeman, Gordon J.
Nadler, Lee M.
Gray, Gary S.

(ii) TITLE OF INVENTION: TUMOR CELLS MODIFIED TO EXPRESS B7-2 AND B7-3 WITH INCREASED IMMUNOGENICITY AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: LAHIVE & COCKFIELD
(B) STREET: 60 State Street, Suite 510
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/101,624;
(B) FILING DATE: 26-JUL-1993;
(A) APPLICATION NUMBER: 08/109,393;
(b) FILING DATE: 19-AUG-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Mandragouras, Amy E.
(B) REGISTRATION NUMBER: 36,207
(C) REFERENCE/DOCKET NUMBER: RPI-008

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 227-7400
(B) TELEFAX: (617) 227-5941

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 107..1093

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CACAGGGTGA AAGCTTTGCT TCTCTGCTGC TGTAACAGGG ACTAGCACAG ACACACGGAT       60

GAGTGGGGTC ATTTCCAGAT ATTAGGTCAC AGCAGAAGCA GCCAAA ATG GAT CCC          115
                                                    Met Asp Pro
                                                      1

CAG TGC ACT ATG GGA CTG AGT AAC ATT CTC TTT GTG ATG GCC TTC CTG          163
Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met Ala Phe Leu
  5                  10                  15

CTC TCT GGT GCT GCT CCT CTG AAG ATT CAA GCT TAT TTC AAT GAG ACT          211
Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe Asn Glu Thr
 20                  25                  30                  35

GCA GAC CTG CCA TGC CAA TTT GCA AAC TCT CAA AAC CAA AGC CTG AGT          259
Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln Ser Leu Ser
                 40                  45                  50

GAG CTA GTA GTA TTT TGG CAG GAC CAG GAA AAC TTG GTT CTG AAT GAG          307
Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val Leu Asn Glu
             55                  60                  65

GTA TAC TTA GGC AAA GAG AAA TTT GAC AGT GTT CAT TCC AAG TAT ATG          355
Val Tyr Leu Gly Lys Glu Lys Phe Asp Ser Val His Ser Lys Tyr Met
         70                  75                  80

GGC CGC ACA AGT TTT GAT TCG GAC AGT TGG ACC CTG AGA CTT CAC AAT          403
Gly Arg Thr Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg Leu His Asn
     85                  90                  95

CTT CAG ATC AAG GAC AAG GGC TTG TAT CAA TGT ATC ATC CAT CAC AAA          451
Leu Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile His His Lys
100                 105                 110                 115

AAG CCC ACA GGA ATG ATT CGC ATC CAC CAG ATG AAT TCT GAA CTG TCA          499
Lys Pro Thr Gly Met Ile Arg Ile His Gln Met Asn Ser Glu Leu Ser
                120                 125                 130

GTG CTT GCT AAC TTC AGT CAA CCT GAA ATA GTA CCA ATT TCT AAT ATA          547
Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Val Pro Ile Ser Asn Ile
```

```
ACA GAA AAT GTG TAC ATA AAT TTG ACC TGC TCA TCT ATA CAC GGT TAC        595
Thr Glu Asn Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile His Gly Tyr
        150                 155                 160

CCA GAA CCT AAG AAG ATG AGT GTT TTG CTA AGA ACC AAG AAT TCA ACT        643
Pro Glu Pro Lys Lys Met Ser Val Leu Leu Arg Thr Lys Asn Ser Thr
    165                 170                 175

ATC GAG TAT GAT GGT ATT ATG CAG AAA TCT CAA GAT AAT GTC ACA GAA        691
Ile Glu Tyr Asp Gly Ile Met Gln Lys Ser Gln Asp Asn Val Thr Glu
180                 185                 190                 195

CTG TAC GAC GTT TCC ATC AGC TTG TCT GTT TCA TTC CCT GAT GTT ACG        739
Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser Phe Pro Asp Val Thr
                200                 205                 210

AGC AAT ATG ACC ATC TTC TGT ATT CTG GAA ACT GAC AAG ACG CGG CTT        787
Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys Thr Arg Leu
            215                 220                 225

TTA TCT TCA CCT TTC TCT ATA GAG CTT GAG GAC CCT CAG CCT CCC CCA        835
Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln Pro Pro Pro
        230                 235                 240

GAC CAC ATT CCT TGG ATT ACA GCT GTA CTT CCA ACA GTT ATT ATA TGT        883
Asp His Ile Pro Trp Ile Thr Ala Val Leu Pro Thr Val Ile Ile Cys
    245                 250                 255

GTG ATG GTT TTC TGT CTA ATT CTA TGG AAA TGG AAG AAG AAG AAG CGG        931
Val Met Val Phe Cys Leu Ile Leu Trp Lys Trp Lys Lys Lys Lys Arg
260                 265                 270                 275

CCT CGC AAC TCT TAT AAA TGT GGA ACC AAC ACA ATG GAG AGG GAA GAG        979
Pro Arg Asn Ser Tyr Lys Cys Gly Thr Asn Thr Met Glu Arg Glu Glu
                280                 285                 290

AGT GAA CAG ACC AAG AAA AGA GAA AAA ATC CAT ATA CCT GAA AGA TCT       1027
Ser Glu Gln Thr Lys Lys Arg Glu Lys Ile His Ile Pro Glu Arg Ser
            295                 300                 305

GAT GAA GCC CAG CGT GTT TTT AAA AGT TCG AAG ACA TCT TCA TGC GAC       1075
Asp Glu Ala Gln Arg Val Phe Lys Ser Ser Lys Thr Ser Ser Cys Asp
        310                 315                 320

AAA AGT GAT ACA TGT TTT TAATTAAAGA GTAAAGCCCA AAAAAAA                 1120
Lys Ser Asp Thr Cys Phe
    325
```

(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 329 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Pro Gln Cys Thr Met Gly Leu Ser Asn Ile Leu Phe Val Met
 1               5                  10                  15

Ala Phe Leu Leu Ser Gly Ala Ala Pro Leu Lys Ile Gln Ala Tyr Phe
            20                  25                  30

Asn Glu Thr Ala Asp Leu Pro Cys Gln Phe Ala Asn Ser Gln Asn Gln
        35                  40                  45

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Glu Asn Leu Val
    50                  55                  60

Leu Asn Glu Val Tyr Leu Gly Lys Glu Lys Phe Asp Ser Val His Ser
65                  70                  75                  80

Lys Tyr Met Gly Arg Thr Ser Phe Asp Ser Asp Ser Trp Thr Leu Arg
                85                  90                  95

Leu His Asn Leu Gln Ile Lys Asp Lys Gly Leu Tyr Gln Cys Ile Ile
            100                 105                 110

His His Lys Lys Pro Thr Gly Met Ile Arg Ile His Gln Met Asn Ser
        115                 120                 125

Glu Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Val Pro Ile
    130                 135                 140

Ser Asn Ile Thr Glu Asn Val Tyr Ile Asn Leu Thr Cys Ser Ser Ile
145                 150                 155                 160

His Gly Tyr Pro Glu Pro Lys Lys Met Ser Val Leu Leu Arg Thr Lys
                165                 170                 175

Asn Ser Thr Ile Glu Tyr Asp Gly Ile Met Gln Lys Ser Gln Asp Asn
            180                 185                 190

Val Thr Glu Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser Phe Pro
        195                 200                 205

Asp Val Thr Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr Asp Lys
    210                 215                 220

Thr Arg Leu Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp Pro Gln
225                 230                 235                 240

Pro Pro Pro Asp His Ile Pro Trp Ile Thr Ala Val Leu Pro Thr Val
                245                 250                 255

Ile Ile Cys Val Met Val Phe Cys Leu Ile Leu Trp Lys Trp Lys Lys
            260                 265                 270

Lys Lys Arg Pro Arg Asn Ser Tyr Lys Cys Gly Thr Asn Thr Met Glu
        275                 280                 285

Arg Glu Glu Ser Glu Gln Thr Lys Lys Arg Glu Lys Ile His Ile Pro
    290                 295                 300
```

```
Glu Arg Ser Asp Glu Ala Gln Arg Val Phe Lys Ser Ser Lys Thr Ser
305                 310                 315                 320

Ser Cys Asp Lys Ser Asp Thr Cys Phe
                325
```

(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1151 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 99..1028

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGAGCAAGCA GACGCGTAAG AGTGGCTCCT GTAGGCAGCA CGGACTTGAA CAACCAGACT      60

CCTGTAGACG TGTTCCAGAA CTTACGGAAG CACCCACG ATG GAC                     104
                                            Met Asp
                                              1

CCC AGA TGC ACC ATG GGC TTG GCA ATC CTT ATC TTT GTG ACA GTC TTG       152
Pro Arg Cys Thr Met Gly Leu Ala Ile Leu Ile Phe Val Thr Val Leu
          5                  10                  15

CTG ATC TCA GAT GCT GTT TCC GTG GAG ACG CAA GCT TAT TTC AAT GGG       200
Leu Ile Ser Asp Ala Val Ser Val Glu Thr Gln Ala Tyr Phe Asn Gly
     20                  25                  30

ACT GCA TAT CTG CCG TGC CCA TTT ACA AAG GCT CAA AAC ATA AGC CTG       248
Thr Ala Tyr Leu Pro Cys Pro Phe Thr Lys Ala Gln Asn Ile Ser Leu
 35                  40                  45                  50

AGT GAG CTG GTA GTA TTT TGG CAG GAC CAG CAA AAG TTG GTT CTG TAC       296
Ser Glu Leu Val Val Phe Trp Gln Asp Gln Gln Lys Leu Val Leu Tyr
                 55                  60                  65

GAG CAC TAT TTG GGC ACA GAG AAA CTT GAT AGT GTG AAT GCC AAG TAC       342
Glu His Tyr Leu Gly Thr Glu Lys Leu Asp Ser Val Asn Ala Lys Tyr
             70                  75                  80

CTG GGC CGC ACG AGC TTT GAC AGG AAC AAC TGG ACT CTA CGA CTT CAC       382
Leu Gly Arg Thr Ser Phe Asp Arg Asn Asn Trp Thr Leu Arg Leu His
          85                  90                  95

AAT GTT CAG ATC AAG GAC ATG GGC TCG TAT GAT TGT TTT ATA CAA AAA       440
Asn Val Gln Ile Lys Asp Met Gly Ser Tyr Asp Cys Phe Ile Gln Lys
     100                 105                 110
```

```
AAG CCA CCC ACA GGA TCA ATT ATC CTC CAA CAG ACA TTA ACA GAA CTG        488
Lys Pro Pro Thr Gly Ser Ile Ile Leu Gln Gln Thr Leu Thr Glu Leu
115                 120                 125                 130

TCA GTG ATC GCC AAC TTC AGT GAA CCT GAA ATA AAA CTG GCT CAG AAT        536
Ser Val Ile Ala Asn Phe Ser Glu Pro Glu Ile Lys Leu Ala Gln Asn
                135                 140                 145

GTA ACA GGA AAT TCT GGC ATA AAT TTG ACC TGC ACG TCT AAG CAA GGT        584
Val Thr Gly Asn Ser Gly Ile Asn Leu Thr Cys Thr Ser Lys Gln Gly
            150                 155                 160

CAC CCG AAA CCT AAG AAG ATG TAT TTT CTG ATA ACT AAT TCA ACT AAT        632
His Pro Lys Pro Lys Lys Met Tyr Phe Leu Ile Thr Asn Ser Thr Asn
        165                 170                 175

GAG TAT GGT GAT AAC ATG CAG ATA TCA CAA GAT AAT GTC ACA GAA CTG        680
Glu Tyr Gly Asp Asn Met Gln Ile Ser Gln Asp Asn Val Thr Glu Leu
    180                 185                 190

TTC AGT ATC TCC AAC AGC CTC TCT CTT TCA TTC CCG GAT GGT GTG TGG        728
Phe Ser Ile Ser Asn Ser Leu Ser Leu Ser Phe Pro Asp Gly Val Trp
195                 200                 205                 210

CAT ATG ACC GTT GTG TGT GTT CTG GAA ACG GAG TCA ATG AAG ATT TCC        776
His Met Thr Val Val Cys Val Leu Glu Thr Glu Ser Met Lys Ile Ser
                215                 220                 225

TCC AAA CCT CTC AAT TTC ACT CAA GAG TTT CCA TCT CCT CAA ACG TAT        814
Ser Lys Pro Leu Asn Phe Thr Gln Glu Phe Pro Ser Pro Gln Thr Tyr
            230                 235                 240

TGG AAG GAG ATT ACA GCT TCA GTT ACT GTG GCC CTC CTC CTT GTG ATG        872
Trp Lys Glu Ile Thr Ala Ser Val Thr Val Ala Leu Leu Leu Val Met
        245                 250                 255

CTG CTC ATC ATT GTA TGT CAC AAG AAG CCG AAT CAG CCT AGC AGG CCC        920
Leu Leu Ile Ile Val Cys His Lys Lys Pro Asn Gln Pro Ser Arg Pro
    260                 265                 270

AGC AAC ACA GCC TCT AAG TTA GAG CGG GAT AGT AAC GCT GAC AGA GAG        968
Ser Asn Thr Ala Ser Lys Leu Glu Arg Asp Ser Asn Ala Asp Arg Glu
275                 280                 285                 290

ACT ATC AAC CTG AAG GAA CTT GAA CCC CAA ATT GCT TCA GCA AAA CCA       1016
Thr Ile Asn Leu Lys Glu Leu Glu Pro Gln Ile Ala Ser Ala Lys Pro
                295                 300                 305

AAT GCA GAG TGAAGGCAGT GAGAGCCTGA GGAAAGAGTT AAAAATTGCT               1065
Asn Ala Glu

TTGCCTGAAA TAAGAAGTGC AGAGTTTCTC AGAATTCAAA AATGTTCTCA GCTGATTGGA     1115

ATTCTACAGT TGAATAATTA AAGAAC                                          1151
```

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 309 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Asp Pro Arg Cys Thr Met Gly Leu Ala Ile Leu Ile Phe Val Thr
 1               5                  10                  15

Val Leu Leu Ile Ser Asp Ala Val Ser Val Glu Thr Gln Ala Tyr Phe
            20                  25                  30

Asn Gly Thr Ala Tyr Leu Pro Cys Pro Phe Thr Lys Ala Gln Asn Ile
        35                  40                  45

Ser Leu Ser Glu Leu Val Val Phe Trp Gln Asp Gln Gln Lys Leu Val
    50                  55                  60

Leu Tyr Glu His Tyr Leu Gly Thr Glu Lys Leu Asp Ser Val Asn Ala
65                  70                  75                  80

Lys Tyr Leu Gly Arg Thr Ser Phe Asp Arg Asn Asn Trp Thr Leu Arg
                85                  90                  95

Leu His Asn Val Gln Ile Lys Asp Met Gly Ser Tyr Asp Cys Phe Ile
            100                 105                 110

Gln Lys Lys Pro Pro Thr Gly Ser Ile Ile Leu Gln Gln Thr Leu Thr
        115                 120                 125

Glu Leu Ser Val Ile Ala Asn Phe Ser Glu Pro Glu Ile Lys Leu Ala
    130                 135                 140

Gln Asn Val Thr Gly Asn Ser Gly Ile Asn Leu Thr Cys Thr Ser Lys
145                 150                 155                 160

Gln Gly His Pro Lys Pro Lys Lys Met Tyr Phe Leu Ile Thr Asn Ser
                165                 170                 175

Thr Asn Glu Tyr Gly Asp Asn Met Gln Ile Ser Gln Asp Asn Val Thr
            180                 185                 190

Glu Leu Phe Ser Ile Ser Asn Ser Leu Ser Leu Ser Phe Pro Asp Gly
        195                 200                 205

Val Trp His Met Thr Val Val Cys Val Leu Glu Thr Glu Ser Met Lys
    210                 215                 220

Ile Ser Ser Lys Pro Leu Asn Phe Thr Gln Glu Phe Pro Ser Pro Gln
225                 230                 235                 240

Thr Tyr Trp Lys Glu Ile Thr Ala Ser Val Thr Val Ala Leu Leu Leu
                245                 250                 255
```

```
Val Met Leu Leu Ile Ile Val Cys His Lys Lys Pro Asn Gln Pro Ser
            260                 265                 270

Arg Pro Ser Asn Thr Ala Ser Lys Leu Glu Arg Asp Ser Asn Ala Asp
        275                 280                 285

Arg Glu Thr Ile Asn Leu Lys Glu Leu Glu Pro Gln Ile Ala Ser Ala
    290                 295                 300

Lys Pro Asn Ala Glu
305
```

(6) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapien
(F) TISSUE TYPE: lymphoid
(G) CELL TYPE: B cell
(H) CELL LINE: Raji

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CCAAAGAAAA AGTGATTTGT CATTGCTTTA TAGACTGTAA GAAGAGAACA TCTCAGAAGT   60

GGAGTCTTAC CCTGAAATCA AAGGATTTAA AGAAAAAGTG GAATTTTTCT TCAGCAAGCT  120

GTGAAACTAA ATCCACAACC TTTGGAGACC CAGGAACACC CTCCAATCTC TGTGTGTTTT  180

GTAAACATCA CTGGAGGGTC TTCTACGTGA GCAATTGGAT TGTCATCAGC CCTGCCTGTT  240

TTGCACCTGG GAAGTGCCCT GGTCTTACTT GGGTCCAAAT TGTTGGCTTT CACTTTTGAC  300

CCTAAGCATC TGAAGCC ATG GGC CAC ACA CGG AGG CAG GGA ACA TCA CCA TCC 353
                  Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser
                                  -30                 -25

AAG TGT CCA TAC CTG AAT TTC TTT CAG CTC TTG GTG CTG GCT GGT CTT    401
Lys Cys Pro Tyr Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu
        -20                 -15                 -10

TCT CAC TTC TGT TCA GGT GTT ATC CAC GTG ACC AAG GAA GTG AAA GAA    449
Ser His Phe Cys Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu
    -5                  1                   5                  10
```

```
GTG GCA ACG CTG TCC TGT GGT CAC AAT GTT TCT GTT GAA GAG CTG GCA    497
Val Ala Thr Leu Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala
                15                  20                  25

CAA ACT CGC ATC TAC TGG CAA AAG GAG AAG AAA ATG GTG CTG ACT ATG    545
Gln Thr Arg Ile Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met
            30                  35                  40

ATG TCT GGG GAC ATG AAT ATA TGG CCC GAG TAC AAG AAC CGG ACC ATC    593
Met Ser Gly Asp Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile
        45                  50                  55

TTT GAT ATC ACT AAT AAC CTC TCC ATT GTG ATC CTG GCT CTG CGC CCA    641
Phe Asp Ile Thr Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro
    60                  65                  70

TCT GAC GAG GGC ACA TAC GAG TGT GTT GTT CTG AAG TAT GAA AAA GAC    689
Ser Asp Glu Gly Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp
75                  80                  85                  90

GCT TTC AAG CGG GAA CAC CTG GCT GAA GTG ACG TTA TCA GTC AAA GCT    737
Ala Phe Lys Arg Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala
                95                  100                 105

GAC TTC CCT ACA CCT AGT ATA TCT GAC TTT GAA ATT CCA ACT TCT AAT    785
Asp Phe Pro Thr Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn
            110                 115                 120

ATT AGA AGG ATA ATT TGC TCA ACC TCT GGA GGT TTT CCA GAG CCT CAC    833
Ile Arg Arg Ile Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His
        125                 130                 135

CTC TCC TGG TTG GAA AAT GGA GAA GAA TTA AAT GCC ATC AAC ACA ACA    881
Leu Ser Trp Leu Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr
    140                 145                 150

GTT TCC CAA GAT CCT GAA ACT GAG CTC TAT GCT GTT AGC AGC AAA CTG    929
Val Ser Gln Asp Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu
155                 160                 165                 170

GAT TTC AAT ATG ACA ACC AAC CAC AGC TTC ATG TGT CTC ATC AAG TAT    977
Asp Phe Asn Met Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr
                175                 180                 185

GGA CAT TTA AGA GTG AAT CAG ACC TTC AAC TGG AAT ACA ACC AAG CAA   1025
Gly His Leu Arg Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln
```

GAG CAT TTT CCT GAT AAC CTG CTC CCA TCC TGG GCC ATT ACC TTA ATC 1073
Glu His Phe Pro Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile
205 210 215

TCA GTA AAT GGA ATT TTT GTG ATA TGC TGC CTG ACC TAC TGC TTT GCC 1121
Ser Val Asn Gly Ile Phe Val Ile Cys Cys Leu Thr Tyr Cys Phe Ala
220 225 230

CCA AGA TGC AGA GAG AGA AGG AGG AAT GAG AGA TTG AGA AGG GAA AGT 1169
Pro Arg Cys Arg Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser
235 240 245 250

GTA CGC CCT GTA TAACAGTGTC CGCAGAAGCA AGGGGCTGAA AAGATCTGAA 1221
Val Arg Pro Val

GGTAGCCTCC GTCATCTCTT CTGGGATACA TGGATCGTGG GGATCATGAG GCATTCTTCC 1281

CTTAACAAAT TTAAGCTGTT TTACCCACTA CCTCACCTTC TTAAAAACCT CTTTCAGATT 1341

AAGCTGAACA GTTACAAGAT GGCTGGCATC CCTCTCCTTT CTCCCCATAT GCAATTTGCT 1401

TAATGTAACC TCTTCTTTTG CCATGTTTCC ATTCTGCCAT CTTGAATTGT CTTGTCAGCC 1461

AATTCATTAT CTATTAAACA CTAATTTGAG 1491

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 288 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly His Thr Arg Arg Gln Gly Thr Ser Pro Ser Lys Cys Pro Tyr
-30 -25 -20

Leu Asn Phe Phe Gln Leu Leu Val Leu Ala Gly Leu Ser His Phe Cys
-15 -10 -5

Ser Gly Val Ile His Val Thr Lys Glu Val Lys Glu Val Ala Thr Leu

```
Ser Cys Gly His Asn Val Ser Val Glu Glu Leu Ala Gln Thr Arg Ile
 15                  20                  25                  30

Tyr Trp Gln Lys Glu Lys Lys Met Val Leu Thr Met Met Ser Gly Asp
                 35                  40                  45

Met Asn Ile Trp Pro Glu Tyr Lys Asn Arg Thr Ile Phe Asp Ile Thr
             50                  55                  60

Asn Asn Leu Ser Ile Val Ile Leu Ala Leu Arg Pro Ser Asp Glu Gly
         65                  70                  75

Thr Tyr Glu Cys Val Val Leu Lys Tyr Glu Lys Asp Ala Phe Lys Arg
     80                  85                  90

Glu His Leu Ala Glu Val Thr Leu Ser Val Lys Ala Asp Phe Pro Thr
 95                  100                 105                 110

Pro Ser Ile Ser Asp Phe Glu Ile Pro Thr Ser Asn Ile Arg Arg Ile
                115                 120                 125

Ile Cys Ser Thr Ser Gly Gly Phe Pro Glu Pro His Leu Ser Trp Leu
            130                 135                 140

Glu Asn Gly Glu Glu Leu Asn Ala Ile Asn Thr Thr Val Ser Gln Asp
        145                 150                 155

Pro Glu Thr Glu Leu Tyr Ala Val Ser Ser Lys Leu Asp Phe Asn Met
    160                 165                 170

Thr Thr Asn His Ser Phe Met Cys Leu Ile Lys Tyr Gly His Leu Arg
175                 180                 185                 190

Val Asn Gln Thr Phe Asn Trp Asn Thr Thr Lys Gln Glu His Phe Pro
                195                 200                 205

Asp Asn Leu Leu Pro Ser Trp Ala Ile Thr Leu Ile Ser Val Asn Gly
            210                 215                 220

Ile Phe Val Ile Cys Cys Leu Thr Tyr Cys Phe Ala Pro Arg Cys Arg
        225                 230                 235

Glu Arg Arg Arg Asn Glu Arg Leu Arg Arg Glu Ser Val Arg Pro Val
    240                 245                 250
```

(8) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1716 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus
(D) DEVELOPMENTAL STAGE: germ line
(F) TISSUE TYPE: lymphoid
(G) CELL TYPE: B lymphocyte
(H) CELL LINE: 70Z and A20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GAGTTTTATA CCTCAATAGA CTCTTACTAG TTTCTCTTTT TCAGGTTGTG AAACTCAACC   60

TTCAAAGACA CTCTGTTCCA TTTCTGTGGA CTAATAGGAT CATCTTTAGC ATCTGCCGGG  120

TGGATGCCAT CCAGGCTTCT TTTTCTACAT CTCTGTTTCT CGATTTTTGT GAGCCTAGGA  180

GGTGCCTAAG CTCCATTGGC TCTAGATTCC TGGCTTTCCC CATCATGTTC TCCAAAGCAT  240

CTGAAGCT ATG GCT TGC AAT TGT CAG TTG ATG CAG GAT ACA CCA CTC CTC    290
          Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu
                -35                 -30                 -25

AAG TTT CCA TGT CCA AGG CTC AAT CTT CTC TTT GTG CTG CTG ATT CGT    338
Lys Phe Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg
            -20                 -15                 -10

CTT TCA CAA GTG TCT TCA GAT GTT GAT GAA CAA CTG TCC AAG TCA GTG    386
Leu Ser Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val
        -5              -1  1               5

AAA GAT AAG GTA TTG CTG CCT TGC CGT TAC AAC TCT CCT CAT GAA GAT    434
Lys Asp Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp
 10                  15                  20                  25

GAG TCT GAA GAC CGA ATC TAC TGG CAA AAA CAT GAC AAA GTG GTG CTG    482
Glu Ser Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu
                30                  35                  40

TCT GTC ATT GCT GGG AAA CTA AAA GTG TGG CCC GAG TAT AAG AAC CGG    530
Ser Val Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg
            45                  50                  55

ACT TTA TAT GAC AAC ACT ACC TAC TCT CTT ATC ATC CTG GGC CTG GTC    578
Thr Leu Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val
        60                  65                  70

CTT TCA GAC CGG GGC ACA TAC AGC TGT GTC GTT CAA AAG AAG GAA AGA    626
Leu Ser Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg
     75                  80                  85

GGA ACG TAT GAA GTT AAA CAC TTG GCT TTA GTA AAG TTG TCC ATC AAA    674
Gly Thr Tyr Glu Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys
 90                  95                  100                 105
```

GCT GAC TTC TCT ACC CCC AAC ATA ACT GAG TCT GGA AAC CCA TCT GCA 722
Ala Asp Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala
110 115 120

GAC ACT AAA AGG ATT ACC TGC TTT GCT TCC GGG GGT TTC CCA AAG CCT 770
Asp Thr Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro
125 130 135

CGC TTC TCT TGG TTG GAA AAT GGA AGA GAA TTA CCT GGC ATC AAT ACG 818
Arg Phe Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr
140 145 150

ACA ATT TCC CAG GAT CCT GAA TCT GAA TTG TAC ACC ATT AGT AGC CAA 866
Thr Ile Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln
155 160 165

CTA GAT TTC AAT ACG ACT CGC AAC CAC ACC ATT AAG TGT CTC ATT AAA 914
Leu Asp Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys
170 175 180 185

TAT GGA GAT GCT CAC GTG TCA GAG GAC TTC ACC TGG GAA AAA CCC CCA 962
Tyr Gly Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro
190 195 200

GAA GAC CCT CCT GAT AGC AAG AAC ACA CTT GTG CTC TTT GGG GCA GGA 1010
Glu Asp Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly
205 210 215

TTC GGC GCA GTA ATA ACA GTC GTC GTC ATC GTT GTC ATC ATC AAA TGC 1058
Phe Gly Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys
220 225 230

TTC TGT AAG CAC AGA AGC TGT TTC AGA AGA AAT GAG GCA AGC AGA GAA 1106
Phe Cys Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu
235 240 245

ACA AAC AAC AGC CTT ACC TTC GGG CCT GAA GAA GCA TTA GCT GAA CAG 1154
Thr Asn Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln
250 255 260 265

ACC GTC TTC CTT TAGTTCTTCT CTGTCCATGT GGGATACATG GTATTATGTG 1206
Thr Val Phe Leu

GCTCATGAGG TACAATCTTT CTTTCAGCAC CGTGCTAGCT GATCTTTCGG ACAACTTGAC 1266

ACAAGATAGA GTTAACTGGG AAGAGAAAGC CTTGAATGAG GATTTCTTTC CATCAGGAAG 1326

CTACGGGCAA GTTTGCTGGG CCTTTGATTG CTTGATGACT GAAGTGGAAA GGCTGAGCCC 1386

ACTGTGGGTG GTGCTAGCCC TGGGCAGGGG CAGGTGACCC TGGGTGGTAT AAGAAAAAGA 1446

GCTGTCACTA AAAGGAGAGG TGCCTAGTCT TACTGCAACT TGATATGTCA TGTTTGGTTG 1506

GTGTCTGTGG GAGGCCTGCC CTTTTCTGAA GAGAAGTGGT GGGAGAGTGG ATGGGGTGGG 1566

GGCAGAGGAA AAGTGGGGGA GAGGGCCTGG GAGGAGAGGA GGGAGGGGGA CGGGGTGGGG 1626

GTGGGGAAAA CTATGGTTGG GATGTAAAAA CGGATAATAA TATAAATATT AAATAAAAAG 1686

AGAGTATTGA GCAAAAAAAA AAAAAAAAAA 1716

(9) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 306 amino acids
(B) TYPE: amino acid
(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ala Cys Asn Cys Gln Leu Met Gln Asp Thr Pro Leu Leu Lys Phe
       -35                 -30                 -25

Pro Cys Pro Arg Leu Ile Leu Leu Phe Val Leu Leu Ile Arg Leu Ser
   -20                 -15                 -10

Gln Val Ser Ser Asp Val Asp Glu Gln Leu Ser Lys Ser Val Lys Asp
 -5              -1  1                5                  10

Lys Val Leu Leu Pro Cys Arg Tyr Asn Ser Pro His Glu Asp Glu Ser
             15                  20                  25

Glu Asp Arg Ile Tyr Trp Gln Lys His Asp Lys Val Val Leu Ser Val
         30                  35                  40

Ile Ala Gly Lys Leu Lys Val Trp Pro Glu Tyr Lys Asn Arg Thr Leu
     45                  50                  55

Tyr Asp Asn Thr Thr Tyr Ser Leu Ile Ile Leu Gly Leu Val Leu Ser
 60                  65                  70                  75

Asp Arg Gly Thr Tyr Ser Cys Val Val Gln Lys Lys Glu Arg Gly Thr
                     80                  85                  90

Tyr Gly Val Lys His Leu Ala Leu Val Lys Leu Ser Ile Lys Ala Asp
             95                  100                 105

Phe Ser Thr Pro Asn Ile Thr Glu Ser Gly Asn Pro Ser Ala Asp Thr
         110                 115                 120

Lys Arg Ile Thr Cys Phe Ala Ser Gly Gly Phe Pro Lys Pro Arg Phe
     125                 130                 135

Ser Trp Leu Glu Asn Gly Arg Glu Leu Pro Gly Ile Asn Thr Thr Ile
 140                 145                 150                 155

Ser Gln Asp Pro Glu Ser Glu Leu Tyr Thr Ile Ser Ser Gln Leu Asp
                 160                 165                 170

Phe Asn Thr Thr Arg Asn His Thr Ile Lys Cys Leu Ile Lys Tyr Gly
             175                 180                 185
```

```
Asp Ala His Val Ser Glu Asp Phe Thr Trp Glu Lys Pro Pro Glu Asp
        190                 195                 200

Pro Pro Asp Ser Lys Asn Thr Leu Val Leu Phe Gly Ala Gly Phe Gly
    205                 210                 215

Ala Val Ile Thr Val Val Val Ile Val Val Ile Ile Lys Cys Phe Cys
220                 225                 230                 235

Lys His Arg Ser Cys Phe Arg Arg Asn Glu Ala Ser Arg Glu Thr Asn
                240                 245                 250

Asn Ser Leu Thr Phe Gly Pro Glu Glu Ala Leu Ala Glu Gln Thr Val
            255                 260                 265

Phe Leu
```

CLAIMS

1. A tumor cell which is modified to express a T cell costimulatory molecule, B7-2.

2. The tumor cell of claim 1 which is transfected with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2.

3. The tumor cell of claim 1 which is stimulated to express B7-2.

4. The tumor cell of claim 1 which has B7-2 coupled to the tumor cell.

5. The tumor cell of claim 1 which expresses a T cell costimulatory molecule, B7.

6. The tumor cell of claim 1 which expresses a T cell costimulatory molecule, B7-3.

7. The tumor cell of claim 1 which expresses an MHC class I molecule.

8. The tumor cell of claim 1 which expresses an MHC class II molecule.

9. The tumor cell of claim 1 which normally expresses an MHC class II associated protein, the invariant chain, and wherein expression of the invariant chain is inhibited.

10. A tumor cell which is modified to express a T cell costimulatory molecule, B7-3.

11. The tumor cell of claim 10 which is transfected with a nucleic acid encoding B7-3 in a form suitable for expression of B7-3.

12. The tumor cell of claim 10 which is stimulated to express B7-3.

13. The tumor cell of claim 10 which has B7-3 coupled to the tumor cell.

14. The tumor cell of claim 10 which expresses a T cell costimulatory molecule, B7.

15. The tumor cell of claim 10 which expresses a T cell costimulatory molecule, B7-2.

16. The tumor cell of claim 10 which expresses an MHC class I molecule.

17. The tumor cell of claim 10 which expresses an MHC class II molecule.

18. The tumor cell of claim 10 which normally expresses an MHC class II associated protein, the invariant chain, and wherein expression of the invariant chain is inhibited.

19. A tumor cell transfected with a nucleic acid encoding a T cell costimulatory molecule, B7-2, in a form suitable for expression of B7-2.

20. The tumor cell of claim 19 wherein the nucleic acid is a cDNA in a recombinant expression vector.

21. The tumor cell of claim 19 further transfected with a nucleic acid encoding a T cell costimulatory molecule, B7, in a form suitable for expression of B7.

22. The tumor cell of claim 19 further transfected with a nucleic acid encoding a T cell costimulatory molecule, B7-3, in a form suitable for expression of B7-3.

23. The tumor cell of claim 19 further transfected with at least one nucleic acid comprising DNA encoding:

(a) at least one MHC class II α chain protein; and

(b) at least one MHC class II β chain protein,

wherein the nucleic acid is in a form suitable for expression of the MHC class II α chain protein(s) and the MHC class II β chain protein(s).

24. The tumor cell of claim 23 which does not express MHC class II molecules prior to transfection of the tumor cell.

25. The tumor cell of claim 19 further transfected with at least one nucleic acid encoding at least one MHC class I α chain protein in a form suitable for expression of the MHC class I protein(s).

26. The tumor cell of claim 25 further transfected with a nucleic acid encoding a β-2 microglobulin protein in a form suitable for expression of the β-2 microglobulin protein.

27. The tumor cell of claim 19 which normally expresses an MHC class II associated protein, the invariant chain, and wherein expression of the invariant chain is inhibited.

28. The tumor cell of claim 27 wherein expression of the invariant chain is inhibited by transfection of the tumor cell with a nucleic acid which is antisense to a regulatory or a coding region of the invariant chain gene.

29. The tumor cell of claim 19 which is a sarcoma.

30. The tumor cell of claim 19 which is a lymphoma.

31. The tumor cell of claim 19 which is selected from a group consisting of a melanoma, a neuroblastoma, a leukemia and a carcinoma.

32. A sarcoma cell which is modified to express a T cell costimulatory molecule, B7-2.

33. The sarcoma cell of claim 32 which is transfected with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2.

34. The sarcoma cell of claim 32 which expresses a T cell costimulatory molecule, B7.

35. The sarcoma cell of claim 32 which expresses a T cell costimulatory molecule, B7-3.

36. The sarcoma cell of claim 32 which expresses an MHC class I molecule.

37. The sarcoma cell of claim 32 which expresses an MHC class II molecule.

38. A composition suitable for pharmaceutical administration comprising an amount of the tumor cells of claim 1 and a physiologically acceptable carrier.

39. A composition suitable for pharmaceutical administration comprising an amount of the tumor cells of claim 10 and a physiologically acceptable carrier.

40. A composition suitable for pharmaceutical administration comprising an amount of the tumor cells of claim 19 and a physiologically acceptable carrier.

41. A composition suitable for pharmaceutical administration comprising an amount of the tumor cells of claim 21 and a physiologically acceptable carrier.

42. A method for treating a subject with a tumor, comprising:

(a) obtaining tumor cells from the subject;

(b) modifying the tumor cells to express B7-2, and

(c) administering the tumor cells to the subject.

43. The method of claim 42 wherein tumor cells are modified by transfection with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2 .

44. The method of claim 42 wherein tumor cells are modified by treatment with an agent which stimulates expression of B7-2.

45. The method of claim 42 wherein tumor cells are modified by coupling B7-2 to the tumor cell.

46. A method of treating a subject with a tumor, comprising:

(a) obtaining tumor cells from the subject;

(b) transfecting the tumor cells with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2; and

(c) administering the tumor cells to the subject.

47. The method of claim 46 wherein the tumor cells are further transfected with a nucleic acid encoding B7.

48. The method of claim 46 wherein the tumor cells are further transfected with at least one nucleic acid encoding at least one MHC class II α chain protein and at least one MHC class II β chain protein in a form suitable for expression of the MHC class II α chain protein(s) and the MHC class II β chain protein(s).

49. The method of claim 46 wherein the tumor cells are further transfected with at least one nucleic acid encoding at least one MHC class I α chain protein in a form suitable for expression of the MHC class I protein(s).

50. The method of claim 49 wherein the tumor cells are further transfected with a nucleic acid encoding a β-2 microglobulin protein in a form suitable for expression of the β-2 microglobulin protein.

51. The method of claim 46 wherein expression of an MHC class II associated protein, the invariant chain, is inhibited in the tumor cells.

52. The method of claim 51 wherein expression of the invariant chain is inhibited in the tumor cells by transfection of the tumor cell with a nucleic acid which is antisense to a regulatory or a coding region of the invariant chain gene.

53. The method of claim 46 wherein the tumor is a sarcoma.

54. The method of claim 46 wherein the tumor is a lymphoma.

55. The method of claim 46 wherein the tumor is selected from a group consisting of a melanoma, a neuroblastoma, a leukemia and a carcinoma.

56. The method of claim 46 wherein the tumor cells are administered by intravenous injection.

57. The method of claim 46 wherein the tumor cells are administered by a route selected from a group consisting of intramuscular injection, intraperitoneal injection and subcutaneous injection.

58. A method for preventing or treating metastatic spread of a tumor or preventing or treating recurrence of a tumor in a subject, comprising:

(a) obtaining tumor cells from the subject;

(b) transfecting the tumor cells with a nucleic acid encoding B7-2 in a form suitable for expression of B7-2; and

(c) administering the tumor cells to the subject.

59. The method of claim 58 wherein the tumor cells are further transfected with a nucleic acid encoding B7.

60. A method of inducing an anti-tumor response by CD4+ T lymphocytes in a subject with a tumor, comprising:

(a) obtaining tumor cells from the subject;

(b) transfecting the tumor cells with at least one nucleic acid comprising DNA encoding:

(i) B7-2,

(ii) an MHC class II α chain protein, and

(iii) an MHC class II β chain protein,

wherein the nucleic acid is in a form suitable for expression of B7-2, the MHC class II α chain protein and the MHC class II β chain protein; and

(c) administering the tumor cells to the subject.

61. A method for treating a subject with a tumor comprising modifying tumor cells *in vivo* to express a T cell costimulatory molecule, B7-2.

62. The method of claim 61 wherein tumor cells are modified *in vivo* by delivering to the subject *in vivo* a nucleic acid encoding B7-2 in a form suitable for expression of B7-2.

63. The method of claim 61 wherein the nucleic acid is delivered to the subject *in vivo* by injection of the nucleic acid in an appropriate vehicle into the tumor.

64. A method for treating a subject with a tumor, comprising:

(a) obtaining tumor cells and T lymphocytes from the subject;

(b) culturing the T lymphocytes from the subject *in vitro* with the tumor cells from the subject and with a stimulatory form of B7-2; and

(c) administering the T lymphocytes to the subject.

TUMOR CELLS MODIFIED TO EXPRESS B7-2 AND B7-3 WITH INCREASED IMMUNOGENICITY AND USES THEREFOR

Abstract

Tumor cells modified to express one or more T cell costimulatory molecules are disclosed. Preferred costimulatory molecules are B7-2 and B7-3. The tumor cells of the invention can be modified by transfection with nucleic acid encoding B7-2 and/or B7-3, by using an agent which induces or increases expression of B7-2 and/or B7-3 on the tumor cell or by coupling B7-2 and/or B7-3 to the tumor cell. Tumor cells modified to express B7-2 and/or B7-3 can be further modified to express B7. Tumor cells further modified to express MHC class I and/or class II molecules or in which expression of an MHC associated protein, the invariant chain, is inhibited are also disclosed. The modified tumor cells of the invention can be used in methods for treating a patient with a tumor, preventing or inhibiting metastatic spread of a tumor or preventing or inhibiting recurrence of a tumor. A method for specifically inducing a $CD4^+$ T cell response against a tumor and a method for treating a tumor by modification of tumor cells *in vivo* are disclosed.

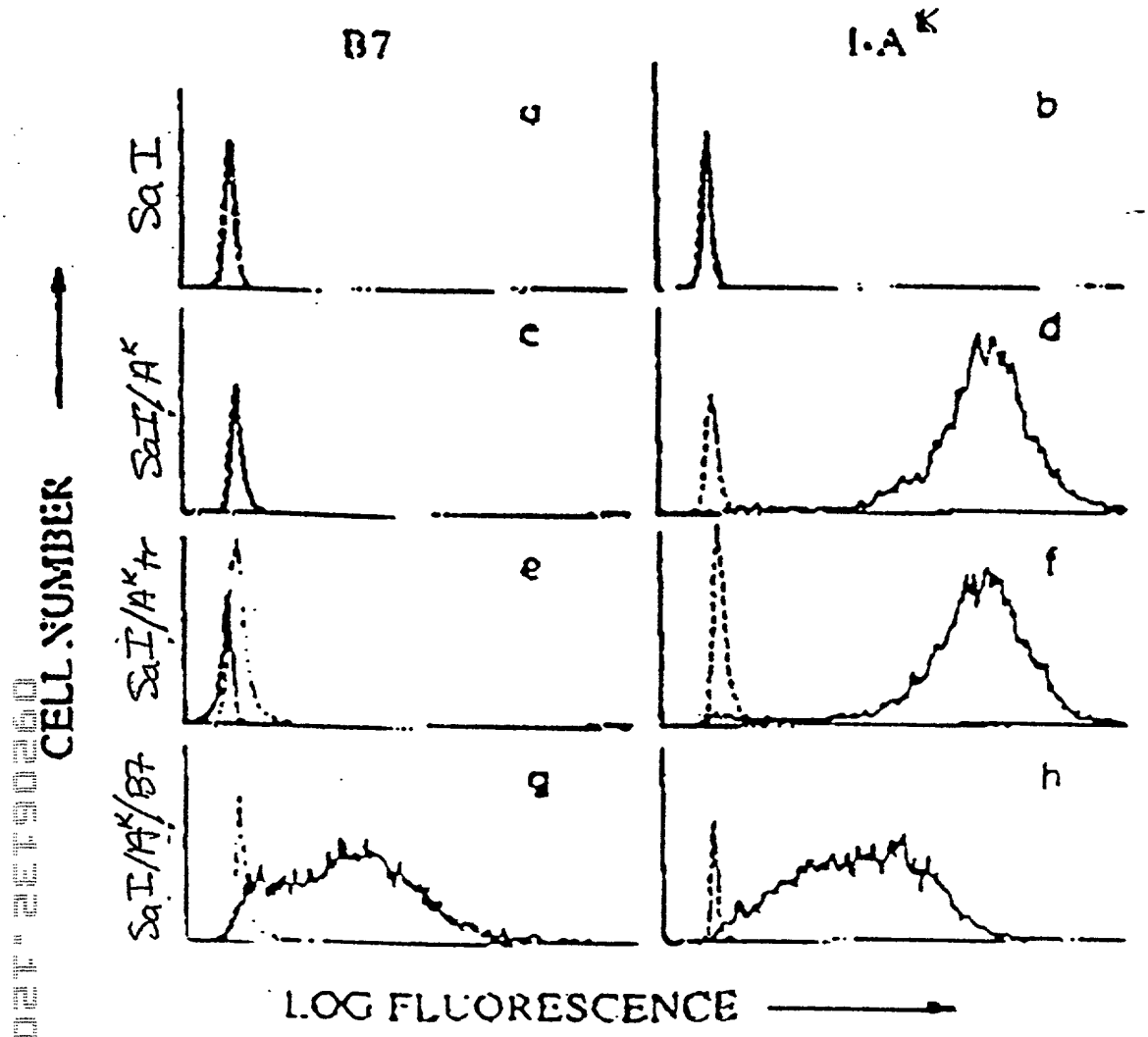
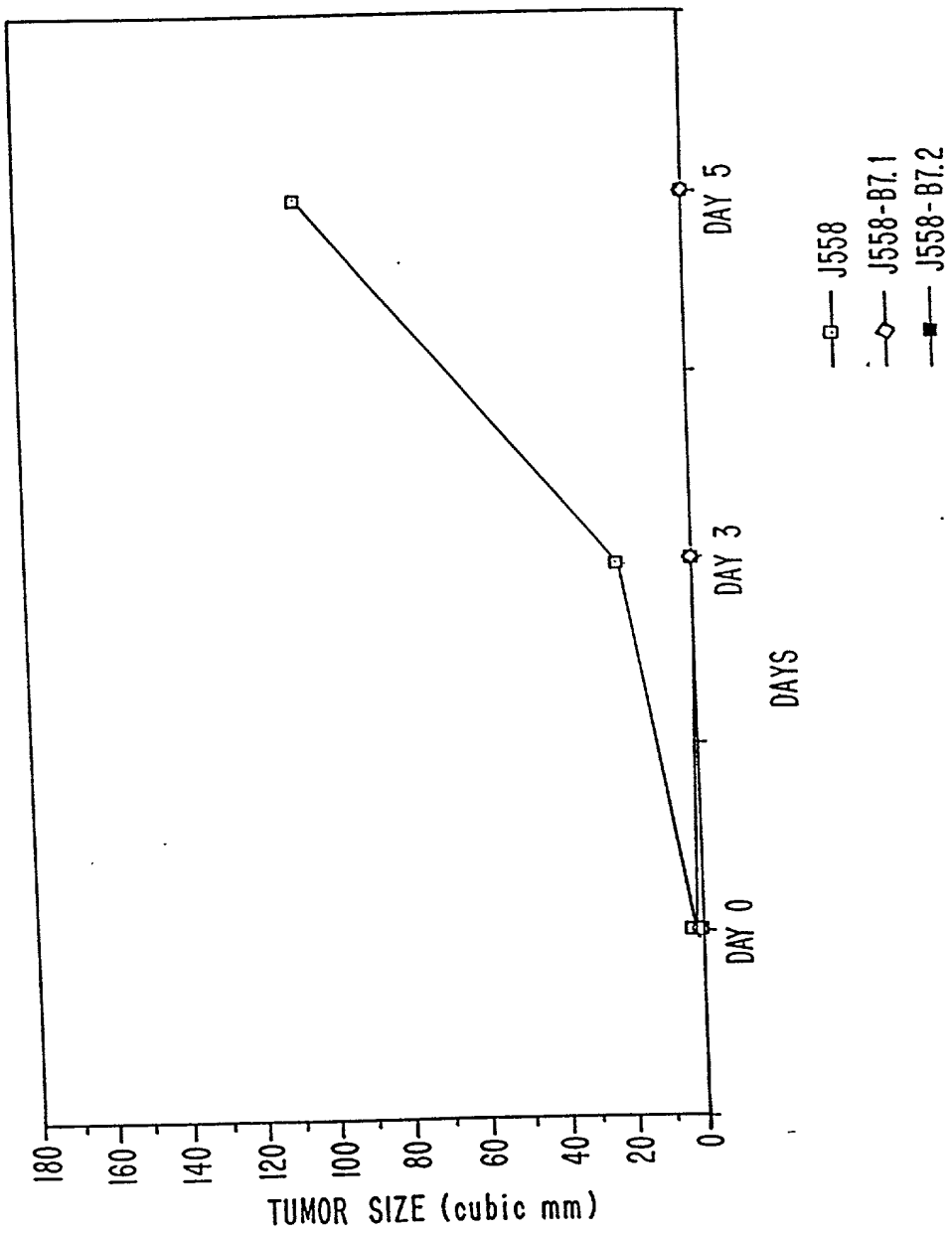

B7
I-A^K
a
b
c
d
e
f
g
h
Sa I
Sa I/A^K
Sa I/A^K
Sa I/A^K/B7
CELL NUMBER
LOG FLUORESCENCE

FIG. 1

FIG. 2


TUMOR SIZE (cubic mm)
180
160
140
120
100
80
60
40
20
0
DAY 0
DAY 3
DAY 5
DAYS
J558
J558-B7.1
J558-B7.2

Attorney's
Docket
Number RPI-008CP

Declaration, Petition and Power of Attorney
for Continuation-in-Part Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

TUMOR CELLS MODIFIED TO EXPRESS B7-2 AND B7-3 WITH INCREASED IMMUNOGENICITY AND USES THEREFOR

the specification of which

(check one)

___ is attached hereto.

X was filed on May 30, 1995 as

Application Serial No. 08/456,104

and was amended on _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

This application in part discloses and claims subject matter disclosed in my earlier filed pending application,

Serial No. 08/147,773, filed November 3, 1993;
Serial No. 08/101,624, filed July 26, 1993;
Serial No. 08/109,393, filed August 19, 1993; and
Serial No. 08/280,757, filed July 26, 1994.

and I hereby claim the benefit of said United States prior application under Title 35, United States Code, §120.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information know to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

AS TO PARENT APPLICATION:

As to the subject matter of this application which is common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to said earlier application, or in public use or on sale in the United States of America more than one year prior to said earlier application; that the common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to said earlier application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on the common subject matter, filed in or designating any country foreign to the United States of America, prior to said earlier application by me or my legal representatives or assigns,

Check one:

_ no such applications have been filed.

X such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
	PCT/US94/08423	July 26, 1994	X Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

AS TO THIS APPLICATION:

As to the subject matter of this application which is not common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application; that said non-common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on said non-common subject matter, filed in or designating any country foreign to the United States of America, prior to this application by me or my legal representatives or assigns,

Check one:

X no such applications have been filed.

_ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John A. Lahive, Jr.	Reg. No. 19,788	Jeremiah Lynch	Reg. No. 17,425
W. Hugo Liepmann	Reg. No. 20,407	Amy E. Mandragouras	Reg. No. 36,207
James E. Cockfield	Reg. No. 19,162	Elizabeth A. Hanley	Reg. No. 33,505
Thomas V. Smurzynski	Reg. No. 24,798	Matthew P. Vincent	Reg. No. 36,709
Ralph A. Loren	Reg. No. 29,325	Paul Louis Myers	Reg. No. 35,965
Thomas J. Engellenner	Reg. No. 28,711	Beth E. Arnold	Reg. No. 35,430
William C. Geary III	Reg. No. 31,359	Anthony A. Laurentano	Reg. No. 38,220
Giulio A. DeConti, Jr.	Reg. No. 31,503	Jane E. Remillard	Reg. No. 38,872
Michael I. Falkoff	Reg. No. 30,833	Jean M. Silveri	Reg. No. 39,030
Ann Lamport Hammitte	Reg. No. 34,858	Mark A. Kurisko	Reg. No. 38,944
John V. Bianco	Reg. No. 36,748	Edward J. Kelly	Reg. No. 38,936

all of: Lahive & Cockfield

and to: Julia D. Hart, Reg. No. 33,132
Dana-Farber Cancer Institute
44 Binney Street
Boston, Massachusetts 02115

Send Correspondence to: Lahive & Cockfield, 60 State Street, Boston, MA 02109

Direct Telephone Calls to: Amy E. Mandragouras, (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor Gordon J. Freeman
Inventor's signature Gordon J. Freeman Date 10 3-95
Residence 305 Walnut Street, Brookline, Massachusetts 02146
Citizenship United States of America
Post Office Address (if different)

Full name of second inventor Lee M. Nadler	
Inventor's signature	Date 10/5/95
Residence 36 Cross Hill Road, Newton, Massachusetts 02159	
Citizenship United States of America	
Post Office Address (if different)	

Full name of third inventor Gary S. Gray	
Inventor's signature	Date
Residence 32 Milton Road, Brookline, Massachusetts 02146	
Citizenship United States of America	
Post Office Address (if different)	

Attorney's
Docket
Number RPI-008CP

Declaration, Petition and Power of Attorney
for Continuation-in-Part Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

TUMOR CELLS MODIFIED TO EXPRESS B7-2 AND B7-3 WITH INCREASED IMMUNOGENICITY AND USES THEREFOR

the specification of which

(check one)

___ is attached hereto.

X was filed on May 30, 1995 as

Application Serial No. 08/456,104

and was amended on _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

This application in part discloses and claims subject matter disclosed in my earlier filed pending application,

Serial No. 08/147,773, filed November 3, 1993;
Serial No. 08/101,624, filed July 26, 1993;
Serial No. 08/109,393, filed August 19, 1993; and
Serial No. 08/280,757, filed July 26, 1994.

and I hereby claim the benefit of said United States prior application under Title 35, United States Code, §120.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information know to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

AS TO PARENT APPLICATION:

As to the subject matter of this application which is common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to said earlier application, or in public use or on sale in the United States of America more than one year prior to said earlier application; that the common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to said earlier application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on the common subject matter, filed in or designating any country foreign to the United States of America, prior to said earlier application by me or my legal representatives or assigns,

Check one:

_ no such applications have been filed.

X such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
	PCT/US94/08423	July 26, 1994	X Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

AS TO THIS APPLICATION:

As to the subject matter of this application which is not common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application; that said non-common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on said non-common subject matter, filed in or designating any country foreign to the United States of America, prior to this application by me or my legal representatives or assigns,

Check one:

X no such applications have been filed.

_ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John A. Lahive, Jr.	Reg. No. 19,788	Jeremiah Lynch	Reg. No. 17,425
W. Hugo Liepmann	Reg. No. 20,407	Amy E. Mandragouras	Reg. No. 36,207
James E. Cockfield	Reg. No. 19,162	Elizabeth A. Hanley	Reg. No. 33,505
Thomas V. Smurzynski	Reg. No. 24,798	Matthew P. Vincent	Reg. No. 36,709
Ralph A. Loren	Reg. No. 29,325	Paul Louis Myers	Reg. No. 35,965
Thomas J. Engellenner	Reg. No. 28,711	Beth E. Arnold	Reg. No. 35,430
William C. Geary III	Reg. No. 31,359	Anthony A. Laurentano	Reg. No. 38,220
Giulio A. DeConti, Jr.	Reg. No. 31,503	Jane E. Remillard	Reg. No. 38,872
Michael I. Falkoff	Reg. No. 30,833	Jean M. Silveri	Reg. No. 39,030
Ann Lamport Hammitte	Reg. No. 34,858	Mark A. Kurisko	Reg. No. 38,944
John V. Bianco	Reg. No. 36,748	Edward J. Kelly	Reg. No. 38,936

all of: Lahive & Cockfield

and to: Julia D. Hart, Reg. No. 33,132
Dana-Farber Cancer Institute
44 Binney Street
Boston, Massachusetts 02115

Send Correspondence to: Lahive & Cockfield, 60 State Street, Boston, MA 02109

Direct Telephone Calls to: Amy E. Mandragouras, (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor Gordon J. Freeman
Inventor's signature . Date
Residence 305 Walnut Street, Brookline, Massachusetts 02146
Citizenship United States of America
Post Office Address (if different)

Full name of second inventor Lee M. Nadler
Inventor's signature Date
Residence 36 Cross Hill Road, Newton, Massachusetts 02159
Citizenship United States of America
Post Office Address (if different)

Full name of third inventor Gary S. Gray
Inventor's signature Date Gary S. Gray August 30, 1995
Residence 32 Milton Road, Brookline, Massachusetts 02146
Citizenship United States of America
Post Office Address (if different)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Gordon J. Freeman, Lee M. Nadler and Gary S. Gray

Serial No.: 08/456,104

Filed: May 30, 1995

For: Tumor Cells Modified to Express B7-2 and B7-3 with Increased Immunogenicity and Uses Therefor

Attorney Docket No.: RPI-008CP

Group Art Unit: 1819

Examiner: K. Hauda

Assistant Commissioner for Patents
Washington, D.C. 20231

ASSOCIATE POWER OF ATTORNEY

Sir:

The undersigned attorney has the power of attorney in the subject application. She hereby grants an associate power to:

Megan E. Williams, Ph.D.
Registration No. P-43,270
Lahive & Cockfield LLPl
28 State Street
Boston, MA 02109

Please continue to forward all written and telephonic communications to Amy E. Mandragouras at the address and telephone number listed below.

Respectfully submitted,

Elizabeth A. Hanley
Registration No. 33,505
Attorney for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
Tel. (617) 227-7400

Dated: June 5, 1998